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VOLUME II

Hydrogen-ion Concentration in Plant Cells and Tissues

by

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PREFACE

Many investigators have published data concerning hydrio concentration, (a) in relation to plant cells and tissues, and (b in relation to the effects upon plants of the reaction of the medium e. g. growth, distribution, germination etc., as affected or apparently affected by pH. The methods used have been very varie and the results have not yet been correlated to any satisfactor degree.

The present monograph is an attempt to bring together th main results concerning the first group of data and to discus the various methods used in the researches. The second grou of data is considered separately in another volume now bein prepared, but it is deemed advisable to limit the contents of thi monograph rather strictly to the details and significance of th internal reaction of plant cells and tissues.

The writer takes this opportunity of recording his gratitud for the invaluable help received, during these investigations an during the writing of this monograph, from his colleagues an students, including Professor T. H. Milroy, Miss M. W. Rea Miss S. H. Martin, Miss M. J. Lynn, Miss M. Clapham, Mr. J. I Armstrong, and particularly Mr. C. T. Ingold. He also take this opportunity of thanking Professor Friedl Weber for th unfailing interest and courtesy, the friendly encouragement an assistance which have brought so many of the workers in thi field into fruitful co-operation.

An index to authors and another to the plants mentione are given at the end, but the need for a subject index has bee met by a fully paginated list of contents which it is believed wil be more helpful as a guide than an alphabetical list of references

Belfast, 10th Feby. 1929

James Small

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PART I

INTRODUCTION

CHAPTER I

THE PROBLEMS

1. PROTEINS. 2. ENZYMES. 3. BUFFERS. 4. SAP, PROTOPLAST AND WALL. 5. VARIATION IN REACTION

The fundamental work of SØRENSEN (1909 sqq.), MICHAELIS (1909 sqq.) and others has made it certain that the concentration of hydrogen ions is one of the important factors in the regulation of enzymic activity. The work of LOEB (1918 sqq., 1922), PAULI (1922) and others has made it just as certain that, in spite of the objections raised by KOPACZEWSKI (1926), reaction is also a very important factor in relation to the characters and activities of proteins *in vitro*. VLÉS (1925) suggests a still greater importance for reaction in relation to the formation and stability of the protein complexes which occur in living protoplasm.

The important regulating power of reaction in enzymic and protein activities having been demonstrated, quite a large number of problems immediately present themselves to the botanical physiologist. Many similar problems have been considered by the zoological physiologists and, so far as the human organism is concerned, many of the problems have been solved more or less satisfactorily.

These problems, in relation to plant cells and tissues, may be conveniently considered under several headings.

1. PROTEINS

The more complex of these ampholytes are known to be relatively easily broken down into simpler substances of a similar nature. One of the first problems which arise is the range of pH within which plant proteins are stable. The range will probably vary with different protein-complexes and may vary, as KOPACZEWSKI (1926) suggests, with the concentration of the substances, with the order and rapidity of the changes in the fluid, with the degree of dispersion of the colloid protein, with the viscosity of the fluid, with the salt content and with other factors.

Within the stable range, changes in the pH may, and according to LOEB and others do, have a considerable effect upon many of the properties of proteins, e. g. water-holding capacity, rate of swelling or imbibition; viscosity; osmotic pressure; conductivity; combination with acids or bases. These effects may reach a maximum around the critical point for ampholytic proteins which is known as the iso-electric point; but two considerations must be emphasized in this connection — 1. that characteristic iso-electric points may be a property *only* of denatured proteins; and 2. that factors other than hydrion-concentration may be so strong that the apparent iso-electric point fluctuates on the pH scale according to the conditions governed by these other factors. LOEB's main thesis concerning protein iso-electric points is regarded by KOPACZEWSKI and some other colloid-chemists as a dangerous simplification. Everyone, however, admits the importance of pH in relation to the properties of proteins.

Many problems then arise concerning the effects of variation in pH upon plant proteins, (a) as part of the living cell and (b) as reserve materials.

Proteins are known to be important constituents of the plasmatic lining of the living vacuolated cell, and the problems involved include the effects of variation in pH on this layer. Permeability to solutes either in the molecular or in the ionic condition, the selectivity of the permeability, the effective osmotic pressure of solutes, the turgidity and growth of the cell, and many other physiological phenomena may or may not be affected by changes in hydrion-concentration, considered as affecting the proteins of the plasmatic lining of the cell.

The nucleus of the living cell and especially the constituent chromosomes are known to be composed largely of protein materials. The problems involved here are of very fundamental importance. By acting upon the nuclear proteins, variations in pH may have an effect upon the vegetative activity of the nucleus, upon nuclear division, upon the chromosomes as regards their individuality, their persistence, their evolutions in mitosis and meiosis, and upon these bodies as the bearers of hereditary factors.

2. ENZYMES

The reaction of the medium is known definitely to be a factor of some considerable importance in controlling the activity of enzymes. Each enzyme shows an optimal reaction and usually also an upper and a lower limit beyond which the enzyme is either inactivated or destroyed. In these characters enzymes differ amongst themselves and even the same enzyme, or rather enzymes with the same action, may be found to differ as regards the optimal pH of the medium when derived from different sources. Further the optimal pH may vary for the same enzyme from the same source when the action of the enzyme is either considered in relation to different characters of the mixed fluid or is taking place in media with different constituents e. g. buffers.

These facts have been determined for the action of enzymes *in vitro*, and they raise various problems in connection with the effect of the internal reaction of plant cells upon the activities of the contained enzymes. That enzymes vary in activity within plants is obvious from the known data concerning germination and other forms of renewed growth and is probable in many other phases of plant life, e. g. storage of reserves etc.

Other problems are then presented for possible solution. What, for example, are the results of reaction effects on enzymes upon the growth, the food production, the respiration and other metabolic activities of the cell? These might well vary from tissue to tissue and be different in chlorenchyma, parenchyma, meristems, stomata, glands, linings of resin canals and gum or oil ducts etc. Variations in pH may be associated with differences in the function or the structure of cells, such as occur in different tissues or in special parts like the pollen grains and embryo sac. Given a number of enzymes in a cell with different optimal reactions, the actual pH might well determine the relative activity of the enzymes and thus direct or control the metabolism of the cell towards a special end-point. Does it?

3. BUFFERS

Assuming the probable importance of reaction and variation in reaction within the various cells of the plant, we must consider the factors governing the reaction. The production of carbonic acid and more complex organic acids is known to be a general feature of metabolism. Katabolism in general consists of oxidation and hydrolysis, the former process being one which usually ends in carbon dioxide and water, after having passed through various stages involving the formation of organic acids. Any control of the internal pH should, therefore, be exerted against this general tendency towards acidification. The utilisation of carbonic acid and organic acids in carbon assimilation is a physiological method of control which might clearly be effective in chlorenchymatous tissues. The building up of proteins from amino-acids is another physiological control which could occur in any tissue but which would be limited to the control of a particular kind of acidification.

Apart from these metabolic controls, there are the *substances* which regulate reaction and which are therefore known as buffers. Buffers or 'moderators' act by removing the active ions from the sphere of action; e. g. by adsorption as in colloid effects, by precipitation as in the case of chalk and calcium oxalate crystals, or by the production of substances which are ionised to a smaller degree as in the case of weakly dissociated acids.

Since the anabolic controls only tend to reduce excess acidity, feeding as it were upon the acidification, they can never involve a positive 'alkalinisation'. The important buffer *substances* in plant cells are, therefore, weakly dissociated acids and calcium salts. These buffers act only within certain limited ranges of reaction and it becomes necessary in the investigation of buffer action to consider not only the buffer effects at reactions which are normal for the cells concerned but also the possible buffer action available against abnormal acidification.

The investigation of buffer action in plants involves determination of

1. the degree of buffer action (i. e. the buffer index) at the natural reaction of the tissues;
2. the degree of buffer action against abnormal pH values which might well be dangerous to the cells concerned;

3. the identification of the buffers acting *at* and *below* the normal pH values;
4. the quantitative estimation where possible of the concentration of the buffer substances identified as present;
5. the elucidation, where possible, of the source of the buffering substances. This source may be direct absorption from the soil, as is possible for phosphates, but it may be metabolic, and dependent upon many physiological factors as in the case of organic acids, like malic and citric acids.

This last point emphasises the importance of metabolic processes as possible factors in the regulation of reaction. They may act directly by removing the acid products as in anabolism or they may act indirectly by the production of weakly dissociated acids as in katabolism. The problems are many and vary with each kind of plant and each variation in the external conditions.

4. SAP, PROTOPLAST AND WALL

The cell sap, being comparatively unorganised and relatively simple, is probably not injured by large variations of reaction. On the other hand reaction may determine to a considerable extent the actual constituents of the sap and so control the food available for the protoplast, as also the osmotic or suction pressure of the sap and therefore the growth of the cell.

The protoplast, regarded as a colloidal complex largely composed of proteins, must be considered as sensitive in a marked degree to large changes of reaction. Whether such large changes are possible without destruction of the protein complexes is exceedingly doubtful and the actual reaction of cytoplasm may be found to show practically no variation, at least within the same cell. There may, however, be highly significant variations in the reaction of cytoplasm as one passes from tissue to tissue or from plant to plant. A cytoplasm which is stable in contact with an external or internal fluid of pH 6·0 may well prove unstable in contact with a fluid of pH 4·0, while another cytoplasm may be quite stable throughout that range. Is there a difference between the pH of the sap and of the cytoplasm? If there is, how is this difference maintained? If there is not, how is the interaction obtained and how do variations affect the pH values of the two kinds of cell content? Many other problems are here raised.

Cellulose walls in general do not show a differentiation in reaction; but collenchyma, calcium pectate or middle lamella; lignified, suberised and cutinised walls, as well as callus plugs and some special cellulosic walls all show what appears to be a definite distinctive and true virage with indicator dyes. The source of this virage and the differentiation which is indicated raises some of the most interesting problems in the study of pH in relation to plants. Does the reaction control the differentiation of cell walls? Do external or internal conditions control this differentiation directly or do they act by controlling the reaction?

5. VARIATIONS IN REACTION

Whether effective buffer action occurs or not, it is conceivable that the reaction of sap, cytoplasm or wall, any or all of these, may remain the same all the time and in all plant cells but it is much more likely to vary. Some of the possible variations in reaction, some or all of which may prove to be significant from the point of view of plant physiology, if not from the purely economic or agricultural point of view; some of these variations may be listed as follows — variations from one part of the cell to another part of the cell; variations within the same cell from time to time; variations during the growth of the plant, diurnal variations, seasonal variations; variation from tissue to tissue, from one part to another of the plant in the same tissue, from plant to plant, from species to species, from genus to genus, from family to family, from group to group, from phylum to phylum; variations with the external medium in lower and in higher plants; variation in connection with definite physiological activities such as photosynthesis, stomatal opening and closing, response to stimuli in taxisms and tropisms. This by no means exhausts the list of possible variations, but it does raise the problems of the connection, causal, correlated or merely concomitant, between any variations in reaction which are found to be associated with any of these differences in times, developments, conditions, functions or phylogenetic positions.

Throughout, of course, there are other effective factors which may either be kept constant and thus eliminated or otherwise taken into consideration. We are aware of such other factors and recognise the importance of some of them. Hydrion concentration is not the master factor of physiology, any more than is temperature, light, humidity or water content.

At present we are considering only the one factor and that only so far as the *internal* reaction of the plant is concerned. The above-mentioned problems are some of the problems involved in the study of hydrion concentration in relation to plant cells and tissues. Having stated our problems we can proceed to describe some of the methods used and some of the results obtained in this study, before re-stating our problems and preparing for further investigations.

PART II

METHODS

CHAPTER II

THE HYDROGEN ELECTRODE

1. PRINCIPLE. 2. MATERIALS. 3. ERRORS

1. PRINCIPLE

The basis of the Hydrogen Electrode Method is NERNST'S theory of electrolytic solution pressure (1889), which states that when metals are immersed in liquids their atoms tend to pass into the ionic state. The EMF of a metal-solution electrode is given by the equation

$$\mathrm{EMF} = \frac{RT}{nF} \ln \frac{P}{p}$$

where R = 8·3 international joules or volt coulombs, T = absolute temperature, n = valency, F = faradays, ln = symbol for Naperian logarithms, P = osmotic pressure and p = electrolytic solution tension.

According to this theory three conditions are possible as the result of placing a plate of metal in a solution containing ions of the same metal. The electrical condition is governed by the relation of the osmotic pressure P to the electrolytic solution tension p. If p be greater than P cations pass from the plate into the solution leaving the plate negatively charged and making the solution positively charged. A potential difference is therefore developed. If p be less than P, cations pass from the solution on to the plate and again a p. d. is developed, this time with the signs of the charges reversed, plate positive and solution negative. If P = p, no potential difference occurs.

Nernst's formula is used in a modified form when applied to hydrogen electrodes, $\frac{C_1}{C_2}$ being substituted for $\frac{P}{p}$, where C_1 and C_2 are the concentrations of hydrogen at the two electrodes. The hydrogen is taken to act just as a metal cation does. Metal-solution or hydrogen-solution potential differences have been taken as proportional to the osmotic pressure of the solution and varying therefore with 'C' the 'effective concentration', i. e. with 'c' the gramme molecular concentration and 'K' the degree of dissociation; so that $C = cK$. The electromotive force of such a system can be calculated and the calculated result has been confirmed experimentally within certain limits. Since these limits vary, all the electrometric methods have been based upon *comparison with a standard*.

The critical point in the theoretical basis of the calculation is the *assumption* that the conductivity per gm. mol. concentration at infinite dilution bears the same relation to the conductivity, per gm. mol. concentration at a definite dilution 'v', as the complete dissociation at infinite dilution bears to the percentage dissociation at dilution 'v'; i. e. that —

$$\frac{\text{conductance at 'v' dilution}}{\text{conductance at infinite dilution}} = \frac{\text{\% dissociation at 'v' dilution}}{\text{complete dissociation at infinite dilution.}}$$

This assumption has been attacked by Lewis (1912) and others, who have substituted $\frac{\text{activity 1}}{\text{activity 2}}$ for $\frac{C_1}{C_2}$ or $\frac{P}{p}$ in the basal formula; the effective concentration of the active ions being supposed to be proportional to the mobility as well as to the concentration of these ions (see Clark 1928). This assumption has also been rejected for living cells by Keller (1912- 1925, see 1925 pp. 22 sqq.), also Gicklhorn (1928) on other grounds. According to Michaelis (1926, p. 128) "the true concentration of the H-ions is, on the other hand, entirely unknown. We have no direct method of measuring it, and probably it is of no great interest to us, for the effectiveness of the H-ions appears to depend only upon the $a[H^+]$, the H-ion activity." Clark (1922) gives various values for the basal potential difference between normal calomel and normal hydrogen electrodes at 25° C. These are 0·2776, 0·2828, 0·3347 and 0·3357, and since they are used as the basis of all hydrogen electrode measurements they have a certain importance. The variation is mentioned here mainly to indicate that insistence on second and third decimal place figures in pH measurements of plant cell-saps is rather academic.

The hydrogen electrode is, for various reasons, neither so useful nor so reliable when applied to living cells as the indicator methods at our present stage of knowledge of reaction phenomena in plants, but for convenience we include a brief summary of the technique.

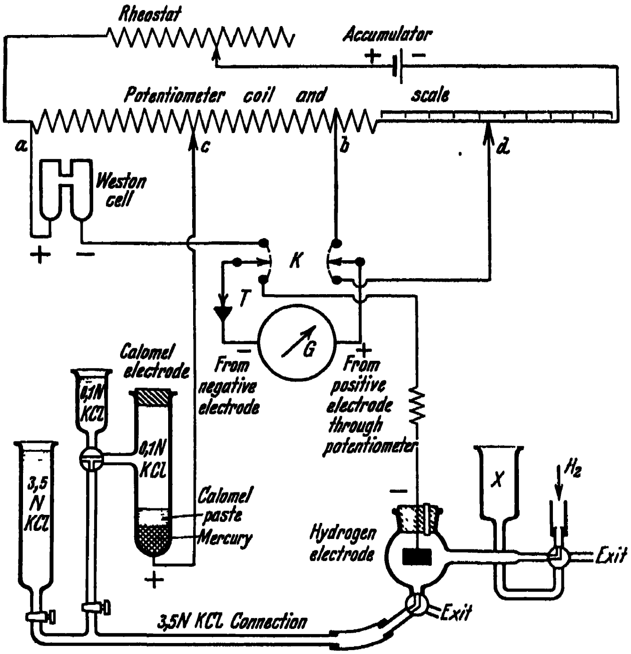

Fig. 1. Hydrogen Electrode Apparatus. — *G* galvanometer; *K* commutator; *T* tapping key (slightly modified ex Milroy after Clark)

A plate of platinum black saturated with hydrogen acts with respect to the hydrogen like a plate of metal. This potential difference can be detected and measured by connecting one such electrode with another similar electrode, if the solutions surrounding the plates be of different concentrations. The solutions are connected by means of a strong solution of potassium chloride in order to eliminate diffusion potential differences at the liquid junctions and the EMF of this concentration cell is measured, usually by means of a potentiometric apparatus.

By experiment it is found that, for all cases where the ion is monovalent and when one of the solutions is ten times more concentrated than the other, this EMF at 18° C is ·0577 volt.

As a matter of convenience a calomel electrode is used instead of the *normal* hydrogen electrode and the EMF between a standard 0·1 calomel electrode and the *normal* hydrogen electrode at 18° C is ·3377 volt. A solution of unknown hydrogen ion concentration is introduced into the hydrogen electrode vessel and the pH is determined by means of the formula —

$$\log_{10}\frac{1}{C_1}\ (\text{or pH}) = \frac{\text{EMF observed} - {\cdot}3377}{{\cdot}0577}.$$

The general apparatus consists of an accumulator, rheostat, galvanometer and potentiometer (Fig. 1). This is calibrated by means of a standard Weston cell. The rheostat is used to bring the fall of potential to ·1 volt per coil of the potentiometer and ·001 volt per cm. along the scaled wire. With a dial potentiometer the dials are set at the reading for the Weston cell (1·0183 volts) and the rheostat adjusted to give no movement in the galvanometer.

In both cases the potentiometer becomes a direct-reading arrangement and the "EMF observed" can be determined for the "unknown" solution. Many details of technique are of importance. They can be found in CLARK's monograph (1928) or in MISLOWITZER's "Bestimmung" (1928).

2. MATERIALS

A large variety of material has been used in hydrogen electrode vessels of the normal type. The external liquid media of plants, e.g. fresh-water, sea-water, soil-water or more commonly aqueous extracts of soil, have been examined. So far as the internal fluids of the plant are concerned, the hydrogen electrode has been used for cell-sap which has been centrifuged out after freezing or withdrawn by special pipettes (WAGNER (1916) or by suction (BENNETT 1927). It has been applied also to an aqueous extract of macerated crushed seeds (NEMEC 1925) and to juice expressed from the whole or part of the plant (by many authors).

The ordinary hydrogen electrode has not been used for cell-wall reactions and obviously cannot be used for the normal living protoplasm of the cell. The external media are expressly omitted from the present volume. The application of the ordinary H-electrode is therefore limited to cell-sap and plant juices. The separation of cell-sap from the cytoplasm by freezing and centrifuging or by the methods of WAGNER or BENNETT may be considered to result in relatively little alteration of the sap, although such alterations may be important in certain cases. These methods also have the advantage of resulting in comparatively slight admixture of the sap with foreign substances, and the disadvantage that unless special precautions are taken the saps from different tissues are obtained as a mixed fluid.

The method of expressing the juice by pressure has none of the advantages of these special methods of sap separation, and this method results in a fluid, 'juice', which is almost certain to contain a smaller or greater quantity of cytoplasmic substance and which in the usual plant material is certain to be a mixture of juices from several different tissues, all possibly with different pH characters, different buffers, etc. The use of expressed juice for pH determinations which are supposed to have some significance in relation to the living cell, can be justified only when quite special nearly homogeneous tissues are used, such as the storage tissue of tubers or the succulent tissue of fruits.

In all these sap or juice materials, the volatile elements of the fluid have an opportunity to escape during the process of preparation. Perhaps the most important and wide-spread 'volatile' element of such liquids is carbon dioxide. It has been demonstrated repeatedly that the carbon dioxide content of the cell may be considerable. It has been definitely shown that the intercellular spaces of plant tissues contain comparatively large percentages of carbon dioxide, up to 21 % in apples, 34 % in potatoes and 28 % in carrots (MAGNESS 1920). It has been shown also that carbon dioxide in similar strength can have a marked effect upon the actual reaction of plant juices (MARTIN 1927—28, INGOLD 1929). The possible importance of the partial pressure of carbon dioxide on the pH of plant juices should, therefore, be recognised by plant physiologists, as it has been almost universally for the human organism by animal physiologists. Since many plant juices are normally of a reaction nearly or quite outside the range in which the carbonic-acid-bicarbonate buffer system is effective, this carbonic acid factor is probably more important in plants than in animals.

3. ERRORS

Apart from these difficulties in the preparation of the fluid to be inserted in the hydrogen electrode vessel, there are errors inherent in the method itself. Firstly, the fluid when introduced must reach equilibrium with the hydrogen saturating the platinum black electrode; that is, before the E.M.F. can be observed the experimental fluid must be reduced. This involves a change in the oxidation-reduction potential of the fluid and probably other properties change at the same time. In fact, the checking of electrometric measurements with colorimetric measurements is definitely advised in certain cases. Electrode 'poisoning' by proteins is another likely error with many plant juices. Toluol and phenol used as preservatives may also interfere with hydrogen electrode determinations.

Oxygen and carbon dioxide occur commonly in plant fluids and both have marked effects. Oxygen naturally interferes with the above-mentioned reduction, until it is removed and the fluid changed at least to that extent. Carbon dioxide dilutes the hydrogen and may also act as an important factor in the acid-base equilibrium, rendering "accurate measurements difficult unless both effects are taken into consideration and put under control" (CLARK 1928, p. 443). The procedure suggested is the determination of the partial pressure of CO_2 in the fluid followed by adjustment of the hydrogen gas with the proper percentage of carbon dioxide or by correction for the known CO_2 pressure of the solution. This carbon dioxide error may be pH 0·5 to pH 1·0, or even more in relatively unbuffered plant fluids.

Concerning the errors of the hydrogen electrode method, *used on plant or animal material*, REISS (1926) p. 83) writes "Cependant cette méthod comporte des causes d'erreur considérables. La destruction probable des complexes, le déplacement des équilibres d'adsorption, la fuite libre de CO_2, le mélange du protoplasme avec les enclaves liquides et autres, le déclanchement de processus fermentatifs, etc., ont pour effet que les résultats obtenus sont — aléatoires." Many criticisms of the indicator methods have been made, but the results have never been described as aleatory! As one definite example we may quote a variation during one determination from pH 5·87 to pH 8·15 found by HEMPEL (1917) in the pitcher fluid of *Nepenthes*, a variation recognised by HEMPEL as due to the effect of the hydrogen of the H-electrode method used.

These and other considerations explain why most of the botanical observations of pH have been made by colorimetric or indicator methods.

CHAPTER III

THE QUINHYDRONE ELECTRODE

1. PRINCIPLE. 2. HYDROQUINHYDRONE ELECTRODE.
3. MATERIALS. 4. ERRORS

1. PRINCIPLE

The quinhydrone electrode has come into use for botanical fluids, mainly because of its great convenience. HABER and RUSS (1904) laid the theoretical foundation of the method, and VALEUR (1900) had described a method of preparing the substance which yields, according to KOLTHOFF (1927), a very pure product giving good results with weakly buffered solutions. BIILMANN (1921 a) introduced the quinhydrone electrode together with another method of quinhydrone preparation (1921 b). The electrode has been investigated from various points of view by the same author (1923, 1924, 1927), GRANGER and NELSON (1921), SØRENSEN (1921), VEIBEL (1923), KOLTHOFF (1923, 1925, 1927), LA MER (1923, 1925), MICHAELIS (1924), ETTISCH (1925), SCHAEFER (1925 26), MISLOWITZER (1925, 1926), KOEHN (1926), SMOLIK (1926), VLÈS (1926), SCHAU-KUANG LIU (1927) and others. Various methods of preparing the quinhydrone are given in the literature[1]), but for general use, it is available ready-made from KAHLBAUM and from the British Drug Houses, London.

In principle this method involves the same phenomena as do oxidation-reduction potentials in general. These are discussed in some detail by CLARK (1928), NEEDHAM (1926) and MISLOWITZER (1928). The essential points may be summarised in the equations —

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[1]) See BIILMANN (1921 b), KOLTHOFF (1925, 1926), MISLOWITZER (1928).

(1) $2H^+ + 2 \text{ electrons} \rightleftharpoons H_2$

(2) $OC_6H_4O + 2 \text{ electrons} \rightleftharpoons {}^-OC_6H_4O^-$

quinone anion of hydroquinone

or

(3) $C_6H_4O_2 + H_2 \rightleftharpoons C_6H_4(OH)_2$

Translating the last equation into electro-chemical terms we have —

(4) $C_6H_4O_2 + 2H^+ - 2F \rightleftharpoons C_6H_4(OH)_2$

The EMF of such a system can be measured as before. Thus for a hydrogen electrode system

$$E = \frac{RT}{nF} \ln \frac{C^1}{C^2}, \text{ which at } 18^\circ C$$

$$= 0{\cdot}00019837 \times 291{\cdot}09 \times \log. \frac{C^1}{C^2} = {\cdot}0577 \log \frac{C^1}{C^2}$$

$$\text{and pH} = \frac{\text{EMF obs.} - {\cdot}3377}{{\cdot}0577}$$

where ·3377 is the EMF of a ·1 N calomel + 1 N hydrogen chain. The potential of the normal hydrogen electrode is ·7042 volt *lower* than a saturated quinhydrone electrode, and ·3377 volt *lower* than a ·1 N calomel electrode, therefore the quinhydrone electrode potential is *higher* than a ·1 N calomel electrode by ·7042 — ·3377 = ·3665 volt.

The relationships may be given thus —

1N $H_2 \longleftarrow$ ·7042 volt $\longrightarrow$ quinhydrone

$\longleftarrow$ ·3377 volt $\longrightarrow$ ·*1N* calomel

therefore ·*1N* calomel $\leftarrow$ ·3665 v. $\rightarrow$ quinhydrone.

Since the calomel electrode is negative to the quinhydrone electrode, instead of being positive as it is with the hydrogen electrode, for a ·1 N calomel saturated quinhydrone system at 18° C the equation for the pH becomes —

$$\text{pH} = \frac{{\cdot}3665 - \text{EMF obs}}{{\cdot}0577}.$$

Kolthoff (1925) gives the corrections for temperature for ·1 N calomel + sat. quinhydrone system thus —

$$\text{pH} = \frac{{\cdot}3665 - {\cdot}00068\ (t - 18) - \text{E obs}}{{\cdot}0577 + {\cdot}0002\ (t - 18)}$$

and for 1·0N calomel + sat. quinhydrone as —

$$pH = \frac{\cdot 4181 - \cdot 00050\ (t - 18) - E\ obs}{\cdot 0577 + \cdot 0002\ (t - 18)}.$$

The great convenience of the quinhydrone method is obvious. It does not involve the preparation of hydrogen, nor the 'blacking' of the platinum electrode. All that is necessary is the usual potentiometric system with a standard Weston cell or other arrangement for calibration, a standard ·1N calomel electrode, and a suitable container for the juice under examination fitted with a (bright) platinum electrode. The juice is placed in the electrode vessel together with sufficient quinhydrone to allow of some being left undissolved. If the liquid is suitable for this type of electrode equilibrium is reached in a very short time and the EMF can be determined directly. The reading must be taken quickly, in a few seconds or at most half a minute, as the potential difference, when the circuit is closed, is of very short duration.

Ettisch (1925) and Samuel (1927) used micro-quinhydrone electrodes. The latter obtained fairly consistent results using a small flat diamond-shaped platinum electrode dipped in quinhydrone powder and inserted in a slit in a piece of tissue, the other end of which was placed in a tube of saturated KCl solution.

2. HYDROQUINHYDRONE ELECTRODE

Sørensen and his collaborators (1921) suggested the use of quinone or hydroquinone in conjunction with quinhydrone in order to avoid certain salt errors. As is indicated below, the salt error of the simple quinhydrone electrode with ordinary plant juices is negligible, but the modification of the electrode into a hydroquinhydrone electrode was again suggested by Billmann and Lund (1923).

When both solid quinhydrone and solid hydroquinone are present in excess in the electrode fluid, the reaction becomes a change from solid hydroquinone to solid quinhydrone, thus —

$$C_6H_4(OH)_2 . C_6H_4O_2 + H_2 \rightleftarrows 2C_6H_4(OH)_2$$

An electrode of this type shows, with a normal hydrogen electrode, an EMF of 0·6179 volt instead of 0·7042. The working equation with temperature corrections becomes

$$pH = \frac{\cdot 2802 - \cdot 00068\ (t - 18) - E\ obs}{\cdot 0577 + \cdot 0002\ (t - 18)}.$$

The only difference in the technique is that solid quinhydrone mixed with an equimolecular weight of solid hydroquinone is added in slight excess to the electrode fluid under investigation.

3. MATERIALS

Quinhydrone in solution dissociates into quinone and hydroquinone. The presence in the electrode vessel of solid quinhydrone yields a saturated solution of the undissociated quinhydrone. But hydroquinone also dissociates, in two stages, the dissociation constants being $K_1 = 1 \times 10^{-10}$ and K_2 lower than 1×10^{-10}, and it is only when $[H^+]$ is greater than 10^{-8} that this dissociation of hydroquinone can be regarded as neglible in calculating the EMF of the electrode. Liquids to be suitable for pH determinations in the quinhydrone electrode must, therefore, have a reaction at least below pH 8.

Further, the hydrogen in an ordinary hydrogen electrode is always at a more or less constant (atmospheric) pressure, and in very accurate work barometric corrections are applied. The relation between EMF and pressure, P, is given by the formula $E = RT \ln \frac{\sqrt{P}}{[H^+]}$. In a reducing medium with P greater than one atmosphere there is an evolution of gas, but with most organic reduction media, including hydroquinone solutions, the hydrogen pressure is so small (of the order of 10^{-22} atmos.) that it can be neglected entirely[1]. The term $\sqrt{P}$ can be eliminated, leaving the E varying with $[H+]$, and so allowing the pH of a solution to be calculated directly from the observed EMF which is given when the solution + reducing medium is connected with an electrode of known potential. From this it follows that liquids which show a measurable pressure of hydrogen are not suitable for pH determinations by means of the quinhydrone electrode. Most plant juices are suitable but many commercial fluids are quite unsuitable and their reaction is measured by other forms of electrodes.

The juices for which the quinhydrone electrode is suitable include those of succulent plants and those of ordinary land plants where the reaction is rarely above pH 7·0. This type of electrode has been used successfully for cactus juice, potato juice,

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1) This cannot be done in the presence of bivalent lead, trivalent iron and similar substances.

etc., **Denny** and **Youden** 1927; **Ohga** 1926; **Ulelha** 1928; **Mason** and **Maskell** (1928); **Ingold** (unpublished work); and others. Its convenience and general applicability merit a wider use in botanical physiology.

4. ERRORS

The quinhydrone electrode, when used for unsuitable fluids is affected by very large errors. **Kolthoff** has shown that by working quickly and using well buffered solutions the results are good up to pH 9, but for ordinary fluids the limit of alkalinity permissible in the quinhydrone method must be taken as pH 8. Within that limit the method avoids some of the errors inherent in the hydrogen electrode method, such as the gradual reduction and the driving off of carbon dioxide. On the other hand the quinhydrone method is, like the indicator methods, subject to errors not inherent in the hydrogen electrode.

It is necessary, for the basal calculation, that quinone and hydroquinone be present in equimolecular proportions, and excess of quinhydrone is added to the solution under investigation as a convenient method of ensuring that the equimolecular proportion is maintained. If, however, salts are present in the solution which act differently upon the quinone and the hydroquinone, then this necessary balance is destroyed. **Sorensen** (1921) demonstrated that chlorides have a much stronger effect upon hydroquinone than upon quinone, and this difference produced alterations in the observed EMF to the extent of 1·3 millivolts for ·5*N* NaCl and 2·8 millivolts for 1*N* NaCl. **Sorensen** and his collaborators suggested the use of a modification of the method whereby this salt error can be avoided, but for plant juices where the salt content is usually much less than ·5 normal concentration, the modification is not necessary on account of the salt error although it may be useful for other reasons. In the case of salts even with ·5*N* NaCl the deviation is only 1·3 millivolts, and, as the deviation varies almost directly with the concentration, in ordinary plant juices this salt error would be quite negligible.

The protein error has been examined by **Kolthoff** (1925), **Mislowitzer** (1926), **Vlès** (1926) and **Schau-Kuang Liu** (1927), who all more or less agree that although there is an error with blood serum, the protein error can be reduced to between ·01 and ·03 pH by dilution of the serum with water to four times the original volume. Since the concentration of proteins in plant juices is normally much lower than that of serum the protein error also may be considered neglible for that material.

A much more serious source of error in the case of plant juices is that shown by the quinhydrone method applied to solutions of phosphates containing glucose. This error is examined by Biilmann and Katagiri (1927), who state that the simple quinhydrone electrode cannot be used for pH determinations of solutions containing glucose and phosphates on account of a specific action of the glucose upon the platinum of the electrode. Since this type of error has been shown to occur in the case of saccharose also (Schreiner 1924), and since mixtures of these sugars with phosphates are comparatively common in plant juices, the sugar and phosphate error must be carefully guarded against in plant juices. Fortunately Biilmann and Katagiri (1927) have examined the possibilities of using the hydroquinhydrone method instead of the simple quinhydrone method, and they came to the conclusion that pH determinations can be made on such sugar and phosphate mixtures by means of the modification known as the hydroquinhydrone electrode and described briefly above. With that electrode system and 10 per cent. glucose in phosphate solutions of various concentrations the deviation is —·140 pH; and with successive dilutions of the glucose by equal volumes of phosphate solution the deviation decreases thus —·073 pH, —·042, —·029 to —·021 pH for ·625 per cent. glucose. The deviation is, therefore, in the second decimal place for 5 per cent. or a lower concentration of glucose, and these results suggest the use of the hydroquinhydrone electrode whenever the plant juices are suspected of containing sugars in strong concentrations.

An alcohol error is described by these same authors, with a deviation of similar magnitude but with an opposite sign. This has little importance in the investigation of botanical fluids but is certainly not negligible with many technical liquids. Dilution to below 5 per cent. alcohol is, however, sufficient to reduce this error to the second decimal place.

The relation of pH to the pressure of carbon dioxide in either of these electrodes is almost linear, just as in the case of the hydrogen electrodes. It is comparatively easy to ensure, by using a closed electrode vessel, that the carbon dioxide content of the electrode fluid is kept as high as it may be when inserted into the vessel, but the process of expressing juice from plant cells certainly disturbs the carbon dioxide balance and this error cannot be completely eliminated by using the quinhydrone type of electrode (cp. Chap. VIII).

The presence of more than a trace of tannin substances in the juices under investigation introduces an error which according to MISLOWITZER (1928 p. 264), can be corrected by determining the pH of the tannin-containing fluid at various dilutions. When such a fluid is diluted the pH *decreases* up to a point, at say 1 : 50, and then increases beyond that dilution. Since the concentration of hydrogen ions in a buffer solution cannot be increased by dilution, the real pH of the fluid is taken as the lowest pH obtained. The difference between this (pH 3·10 at 1 : 50 dilution) and the original undiluted solution (pH 3·80) was ·70, indicating that a tannin error of that extent can be corrected by the method of successive dilutions.

In the examination of acid plant juices by means of electrodes of the quinhydrone type, it is necessary to consider the possible presence and the effect of sugars with phosphates, tannins, and carbon dioxide. The great advantages of such methods lie in their rapidity and simplicity.

CHAPTER IV

MICRO-HYDROGEN ELECTRODES

1. PRINCIPLES. 2. MATERIALS. 3. ERRORS

1. PRINCIPLES

Micro-electrodes have been used in many investigations by plant physiologists e.g. Bose, Small, Gelfan, Brooks and Gelfan and others (see Keller and Gicklhorn 1928); but the coating of a metal point with platinum black and the saturation of such a minute electrode with hydrogen involve a technique which has developed only recently.

These micro-hydrogen electrodes are a natural development from the standard types. They are described and figured in some detail by Mislowitzer (1928). Briefly, the main principle of most patterns may be described as a reduction in the size of that part of the hydrogen-electrode apparatus which contains the platinum-black plate. McClendon's blood electrode-vessel has a capacity of 2cc., and the same worker designed another to be swallowed for testing gastric juice. Bodine and Fink (1925) carried the reduction to 0·015 to 0·02cc. and Bodine (1927) to 0·01cc., with a fine adjustment for the metal point. Lehmann (1923) while reducing the volume of liquid to a drop upon a glass table retains a relatively large space for hydrogen. Radsimowska (1924) has a somewhat similar design. Winterstein (1927) reduces all the fluids and spaces almost to capillary dimensions, thus eliminating the so-called 'dead spaces' of the apparatus. Ettisch (1925) has described a variant using quinhydrone fluids. Sannié (1924) and Solowiew (1926) describe similar arrangements.

As applied to cells and tissues, all these models present manipulative difficulties and they are applicable only to sap or juice *in vitro*. Schade, Neukirch and Halpert (1921) used a glass tube drawn to a fine point with an adjustable metal electrode within the tube. This was used for sub-cutaneous measurements on human subjects. These workers realised the importance of the carbon dioxide factor and used hydrogen plus 5·6 % carbon dioxide for the saturation of the electrode. **Taylor** and **Whitaker** (1927) describe and figure an improved micro-hydrogen electrode of a type essentially similar to that of **Schade** but with refinements, particularly in the adjustment of the metal point within the sheathing quartz pipette and in the sealing of the joints. The apparatus is designed to be used with a micro-manipulator and dead spaces are reduced to a minimum.

2. MATERIALS

These two micro-hydrogen-electrodes are expressly designed for use with living cells and tissues. The **Schade** model has been used on human victims, while the **Taylor-Whitaker** model has been used on *Nitella* but is designed for general use in living cells and tissues.

3. ERRORS

Applied to plant material in the form of the large cells of *Nitella*, the last type of electrode was found to show a distinct difference in its behaviour in relation to cell sap and to protoplasm. The pH of the pure sap could be determined readily and with consistent results so long as the small quantity of fluid which is drawn into the point of the micropipette did not contain any protoplasmic granules. The presence of even a few such particles had a marked effect upon the potential recorded. So much so that, although the average of all their readings was pH 5·47, these authors "are inclined to regard the higher value (pH 6·16) as the more reliable" — because this was obtained in the case of one cell where the cell sap was known to be free from protoplasmic mixture. As this method involves mechanical penetration of the cell, manipulation of the contents, and the treatment of these contents chemically with hydrogen, a considerable degree of error might be expected; but when we find an experimental variation of at least 0·7 in the results obtained with all the difficulties of micromanipulation, the writer is distinctly of the opinion that, so far as sap is concerned, the R. I. M. or even a drop-indicator method is preferable, not only as easier to apply but also as much more likely to give consistent and reasonably accurate results.

The advantages of the use of this micro-hydrogen electrode would, therefore appear to be confined to measurements of the pH of protoplasm. But here we can quote the authors *in extenso*. "The protoplasm instantly produces a potential of between + ·090 and + ·030 volts with respect to hydrogen zero . . . It is clear that these potential readings cannot be regarded as representative of the concentration of hydrogen ions in the protoplasm". It is concluded that these data indicate the oxidation-reduction potential of protoplasm. "In order to obtain a pH measurement of the protoplasm it would probably be necessary first to completely overcome the buffer action by wholly reducing the protoplasm with hydrogen. But if this were done it could hardly be expected that the value obtained would be that of normal, living protoplasm".

There is thus a very large error involved in the measurement of protoplasmic pH by means of the hydrogen electrode, attributed to a 'poising' action by TAYLOR and WHITAKER, and possibly to the formation of a film or membrane around the electrode by HEILBRUNN (1928). The latter mentions an antimony electrode (developed by UHL and KESTRANEK, KOLTHOFF, VLÈS and VELLINGER, see CLARK 1928), and designed as a micro-electrode by BUYTENDIJK and WOERDEMAN (1927). This type has been applied to "frogs' eggs and similar objects," and appears to give somewhat more reasonable results but, according to HEILBRUNN "there is no means of knowing whether or not they are accurate". In any case this does not seem to have been applied to plant material. The antimony electrode requires calibration with buffered solutions and CLARK (1928 p. 427) quotes ROBERTS and FENWICK (MS) on other points, of which the source of error in the presence of dissolved oxygen and the accuracy of the method provided that "equilibrium is approached from the alkaline side", are the most important from the point of view of plant cells and tissues, where dissolved oxygen is frequently present and where the sap is usually acid.

CHAPTER V

COMPARATOR INDICATOR METHODS

1. PRINCIPLES. 2. MATERIALS. 3. ERRORS

The variations in the end points of an acid-base titration with methyl-orange and with phenol-phthalein as indicators is well known, as is also the disturbing effect of free carbon dioxide on a titration of carbonate with acid, using litmus as an indicator. These indicators used to be described as slightly dissociated acids or bases which differed in colour from their salts, but the more generally accepted view is that an indicator consists of isomeric forms which tend to assume an equilibrium condition (i. e. of tautomers). One of these tautomers is dominant when the indicator is undissociated and the other when the indicator is highly dissociated. Recent experiments (see below under lipoid error) indicate the possible existence of more than two tautomers in at least one indicator.

The number and variety of these substances which 'indicate' has been largely increased since 1910 and it has become important to choose from a plethora of indicators those which are as little liable as possible to the specific errors of indicators.

1. PRINCIPLES

The principles underlying the application of indicators are fully discussed by MICHAELIS (1926), KOLTHOFF (1926), MISLOWITZER (1928), CLARK (1928) and others. In general each indicator is a more or less pure colour (one or other tautomer predominating) outside its so-called 'useful' range, while within that range the tautomers behave as in the usual acid-base equilibria. The relative proportions of the tautomers of different colours give a graded series of tints at the various hydrion concentrations within the 'useful' range. Methyl red, for example, is practically pure yellow above pH 6·0 and practically pure red below pH 4·4, between these points the mixture of yellow and red give yellowish orange, orange, pinkish-orange, pale pink and grades of deeper pink, so that by carefully comparing the tint given by a solution of unknown pH with that given by the same concentration of indicator in a series of solutions of known hydrion concentrations the pH can be determined to the first decimal place.

Since both quality and depth of tint are considered it is clear that the concentration of the indicator is important, as well as the depth of the layer of coloured fluid through which the virage is obtained. The importance of this latter point is emphasised in the case of the dichromatism of brom-phenol blue. The indicator may appear blue in a thin layer and red when viewed through a thick layer. In the same way a solution which appears blue in daylight may appear red in electric light[1]).

The normal procedure is to set up a series of solutions of definite pH, strongly buffered so that the pH is not readily affected, and to use these solutions as standards for comparison with similar volumes of unknown fluids to which have been added the *same quantity of indicator* as was added to the standards. The standard solutions and the fluids under investigation *must be placed in containers of exactly the same kind* so that the same thickness of fluid is viewed in each case.

The normal comparator method involves the use of standard test-tubes, in each of which from 5cc. to 20cc. of fluid is placed. In order to ensure the viewing of the same depth of fluid, the tubes are placed in some kind of a frame (called a comparator) so that the virage is obtained through a uniform part of the tube. There are at least three openings; in the centre one is placed the fluid to be determined, and then the tint given by that fluid *plus* a definite number of drops of the indicator is compared on either side with the standard tints.

Clark (1928 p. 120) suggests the possible use of uniform long homeopathic vials; the same author also gives a very useful colour chart which for rough work may be substituted for the fluid colour standards. Flat-sided 'spectrum' bottles are accurate

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1) See Clark 1928 pp. 161—164.

and useful for small quantities of fluid. These have been used by MARTIN (1927) and others.

In addition to these methods of comparison, various other methods have been used by means of which the preparation of buffered solutions of accurately known pH is avoided. The chief of these is known as GILLESPIE's drop-ratio method. In this case the fully transformed indicators are used in two tubes, one containing the alkaline form and the other the acid form. The dissociation curve of the indicator is utilised for the determination of how many drops of indicator should be added to each tube in order to obtain a combination of the colours in the proper proportions for a definite pH value; the combined virage being obtained by viewing the two tubes together in a double comparator. This method with various refinements is fully described by CLARK (1928). A similar method has been devised by MICHAELIS (see CLARK p. 127) using indicators which have a coloured and a colourless form like phenol-phthalein, e. g. the nitrophenol series.

Two-colour indicators, fully transformed into alkaline and acid forms, have also been used in the form of double colour-wedges which when calibrated have been quite as accurate as GILLESPIE's drop-ratio method. These colour wedges were used by BJERRUM (1914), BARNETT (1921), MYERS (1922), KOLTHOFF (1924), VLÈS (cited by REISS 1926), MCCRAE (1926), WHERRY (cited by CLARK p. 170) and others. Tintometer glasses (SONDÉN 1921) have also been used, as have coloured inorganic solutions, (KOLTHOFF 1922).

All these methods are alike in that they involve the comparison of the virage of a comparatively *large* quantity of experimental fluid with that of a standard.

2. MATERIALS

These comparator methods have been used on much the same materials as are investigated by means of the hydrogen-electrode. In so far as plant cells and tissues are concerned, the large quantity of fluid required confines the use of these methods to plant juices obtained by pressure or other means and to other fluids, such as the sap from the wood, which can be obtained in relatively large volume.

3. ERRORS

The errors inherent in the preparation of such large volumes of sap material have been indicated already under 'Materials' in Chapter II. There are the same difficulties in preparing a sample for comparator determinations as for investigation by the hydrogen electrode.

On the other hand, the specific errors of the hydrogen-electrode, i. e. the reduction error, electrode poisoning, oxygen error, carbon-dioxide-dilution error, and errors involved in the preparation of the electrodes and the hydrogen, all these are eliminated by the use of indicators, as are also the phosphate-sugar and tannin errors of the quinhydrone electrode.

The errors inherent in the use of comparator indicator methods may be grouped thus: —

(a) self-colour of the solutions,
(b) very dilute or unbuffered solutions,
(c) salt error,
(d) protein error,
(e) temperature error,
(f) other errors.

Some of these are discussed by Clark (1928, Chap. VIII) who gives convenient tables for reference. The following account is, however, based mainly on Kolthoff's monograph on indicators (1926), also Reiss (1926) and Pfeiffer (1927).

(a) *Self-colour of the Solutions.* — This error may be corrected either by Sørensen's method of colouring the standard buffer solutions to match the solution under investigation (see Clark, 1928, p. 171), or by using a six-hole double comparator with a tube of the self-coloured fluid behind each of the two tubes of standard buffer-fluid *plus* indicator and a tube of plain water behind the tube of self-coloured fluid *plus* indicator (*ibid*).

(b) *Very dilute solutions.* — With an acid indicator having a dissociation constant of 10^{-8} in the usual concentration of about 10^{-6} molar, neutral water *appears* to have a pH of 6·85 instead of 7·00, an error of 0·15. Most of the indicators commonly used have a dissociation constant of less than 10^{-8} and would give an error much smaller than 0·15. Most of the solutions dealt with in plants have a concentration considerably greater than 10^{-6}, and this would normally reduce the error to the second decimal place and render it negligible. Methyl orange and phenol phthalein, however, may give much larger errors; the latter, for example, may indicate a pH 8·7 in a solution of pH 10, when 0·1 cc. 1 % phenol phthalein is added to 10 cc. of 0·0001 N NaOH. The methyl orange error in very dilute acid solutions is considerably smaller than this if the indicator be used in the normal dilution.

(c) *The salt error.* — With concentrations below 1·0 N of neutral salts such as KCl and NaCl this error is at most 0·06 pH for tropaeolin 00 and thymol blue; 0·08 pH for methyl orange and methyl yellow; rising to — 0·35 pH for 0·5 N KCl, with a larger error at very small salt concentrations, for bromo-phenol blue which is therefore not suitable for very dilute solutions; —0·55 pH for p-nitrophenol, another 'very suitable' indicator; —0.15 pH for phenol red and +0·12 pH for neutral red. These errors become significant in dealing with sea-water. Kolthoff recommends amongst others for use with very dilute solutions of electrolytes the following indicators — cresol red, neutral red, B.T.B., B.C.P., M.R. and methyl orange. The 'salt error' of these at low electrolyte content is small.

(d) *The protein error.* — On account of the ampholytic nature of proteins, Sorensen and others have shown that most azo-dyes and congo-red should be completely avoided in pH measurements of protein-containing media. The protein error of methyl red in a mixture of 2 per cent. egg albumin and HCl may be as much as 0·26 pH at pH 5·53; thus electrometrically it may be pH 5·53 while colorimetrically pH 5·27 is indicated. Where only the decomposition products of proteins are present and no undecomposed protein, the error with methyl red is confined to the second decimal place, but hydrolysed serum may give a methyl red protein error of pH 1·1 greater by the colorimetric method. Reiss (1926 p. 64) found that gelatin fragments in an aqueous medium gave pH indications with various indicators which differed by ± 0·5 to ± 1·0 from that of the fluid around the particles, and tentatively compares this with the protoplasm and the cell sap. Clark (1928 p. 185—186) quotes tables of protein errors by Cohen and others.

(e) *The temperature error.* — The dissociation constants of indicators, like those of other electrolytes, vary with the temperature but the change is very slight at ordinary room temperature variation in temperate regions. For example, for the change between 18° and 70° KOLTHOFF gives values around 0·2 to 0·7 for the usual indicators and the temperature coefficient for the change in phenol phthalein is given as 0·01 per degree, the correction being subtracted for the temperatures above 18° C.

(f) *Other errors.* So far as comparator methods are concerned these are mainly due to lipoid action and chemical changes. *The action of lipoids.* — REISS and PFEIFFER cite FAURÉ-FREMIET for the statement that indicators in lipoid solution take the colour of the undissociated form. There are, however, several interesting and easily conducted experiments, which can be done with methyl red, diethyl red, dilute acid or dilute alkali and various oily substances. The combination of indicator and dilute acid or alkali[1]) is shaken up with the oily liquid in a test-tube and set aside until separation has taken place. With *clove* oil, consisting mainly of the liquid phenol eugenol, the oily layer takes up the indicator in the *red or yellow form* according to the reaction of the aqueous liquid. With *liquid paraffin* a yellow form is taken up from the red aqueous acid liquid and no colour at all from the yellow aqueous alkaline liquid, thus suggesting the existence of *two* yellow forms of DER, one soluble and the other insoluble in liquid paraffin[2]). The theoretical aspects of this phenomenon are interesting in relation to the theories of indicators.

With olive oil, free oleic acid[3]) and castor oil, true lipoids, the neutral *orange* tint occurs in the oily layer above the aqueous liquid whether the latter be red or yellow. REISS (1926) found that particles of mastic behaved in the same way as do the true lipoids, taking the neutral form always. The presence of a 'neutral' tint with DER and MR is, therefore, not to be taken as necessarily an indication of true neutrality with lipoids. With an oily substance like liquid paraffin the virage may or may not be true, while with an oily substance like clove oil the virage depends upon the pH of the circumambient fluid. With liquid paraffin and phenol phthalein the red form is not taken up by the paraffin. With brom-cresol green the blue form is not taken up by castor oil but the yellow form with an acid aqueous layer shows in the oily fluid.

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[1]) Or slightly alkaline tap water in the case of castor or olive oil, in order to avoid saponification.

[2]) The acid forms of methyl red and diethyl red which are *red* in *aqueous* solutions may, however, be *yellow* when in solution in *liquid paraffin.*

[3]) This and other fatty acids are yielding very interesting results in a systematic investigation now in progress.

Chemical change of the indicators. — Reduction, producing considerable changes in the indicators, interferes with many dyes particularly Nile blue and cresyl blue, but the usually selected indicator dyes are not affected to any inconvenient extent by this error.

Considering all these errors in relation to plant fluids —

(a) We may correct the 'self-colour' error by the double comparator method or avoid it by the use of capillator methods described in the next chapter.

(b) We may neglect the 'dilute solution error' if the methyl orange and phenol phthalein types of indicator be avoided.

(c) With the usually selected indicators the 'salt error' amounts to about ± 0·1 in the pH and that is the general limit of accuracy in determinations of the pH of plant juices by means of indicators.

(d) The 'protein error' varies considerably with the material used, but for plant juices where the protein content is relatively small the correction is usually of the same magnitude as the salt error or smaller. As the protein correction is more usually of a + sign and the salt correction more usually a minus sign these two errors may well cancel each other in the common plant juices.

(e) The 'temperature error' may become of importance in the tropics but with our usual variations of 15° C to 25° C in room temperatures, it seldom reaches the first place of decimals and may conveniently be neglected except during a really hot summer.

(f) The 'errors' in this group require not so much correction which is difficult to apply, as careful attention to detail in the interpretation of the results obtained.

CHAPTER VI

CAPILLATOR INDICATOR METHODS

1. PRINCIPLES. 2. MATERIALS. 3. ERRORS

The necessity for a comparatively large volume of fluid for a pH determination by the various comparator methods and the frequency with which only a small volume of such fluid is available have led to the adoption of various alternative methods, amongst which the capillator method stands out as most generally useful and reliable.

1. PRINCIPLES

Capillary tubes were used by IRWIN (1925) for comparison of dye concentrations. WALTHER and ULRICH (1926) used capillary tubes 5—7 mm. long in which buffered indicator fluids were compared with the fluid of unknown pH plus indicator. These capillary tubes were then viewed along their length in order to get depth of colour for comparison. CLARK (1928 p. 139) quotes NEEDHAM (without reference) and RAPKINE (cited 'personal communication') as using capillary tubes. The B.D.H. capillator has, however, been readily obtainable since 1925 from the British Drug Houses Ltd., and has been used in this department for several years with increasing satisfaction in the results obtained. This method is, according to TUSTING COCKING (1926) due to H. A. ELLIS, Capillator Patent No. 235 458 (1924).

The capillator outfit (fig. 2) consists of a set of carefully gauged alkali-free glass capillary tubes to each of which an appropriately small size of rubber bulb can be attached for taking in or ejecting the fluids, (2) a series of indicator solutions made slightly stronger than usual, (3) a series of "capillator cards" consisting of buffered indicator solutions sealed in capillary tubes, mounted in sets of three tubes for each step of 0·2 in pH throughout the ranges for each indicator. The sets of capillary tubes are mounted on white cards with a slit for comparisons by transmitted light, in addition to those by light reflected from the white card. Capillator series from pH 4 to pH 11 are available.

A very small quantity (one drop) is drawn into the micro-pipette (capillary tube plus rubber bulb), so that the tube is filled to the single graduation. This fluid is transferred to a very small watch-glass provided for the purpose. Without rinsing the micro-pipette is filled again to the mark with the fluid under investigation, and this fluid is mixed in the watch-glass with the indicator. The mixed liquid is then drawn into the micro-pipette and compared with the Capillator standards. The pH can then be placed at one or between two of the triple sets of standard tints. In this way the pH of a fluid is easily determined, with reasonable accuracy to a single unit in the first decimal place.

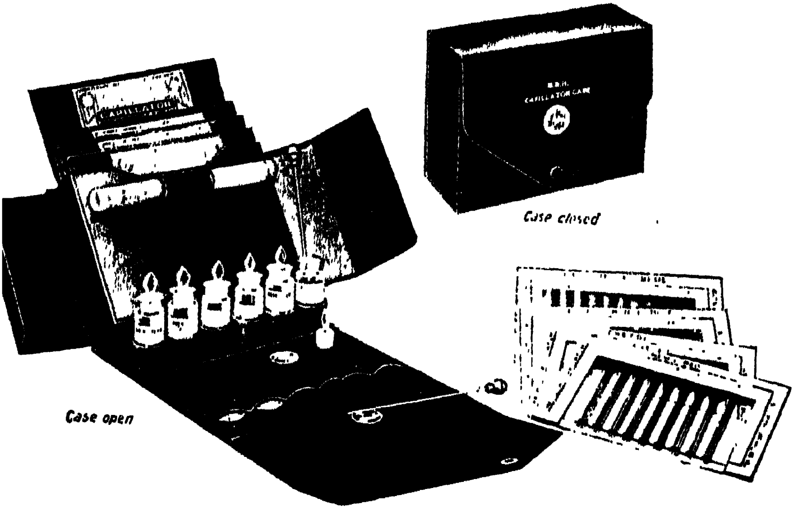

Fig. 2. B. D. H. Capillator Case

The principle of this B.D.H. capillator method is fundamentally the same as that of the comparator with important modifications in the quantity of fluid required and in the greater concentration of indicator which makes comparison much easier than in Walther and Ulrich's method. The use of three tubes for each tint renders any change in the standard obvious at once, since all three tubes seldom or never change at the same speed.

2. MATERIALS

The capillator method is of general applicability and, with buffered fluids, can be used for any investigation in which the standard comparator method is applicable; it has a number of advantages over that method. It is simple, convenient and rapid, and might well replace GILLESPIE's method with its various modifications where the task of making up buffered indicator standards is avoided for one reason or another.

For titrations where a series of points are required, as in the determination of titration curves for buffer indexes, the capillator is very serviceable, since the small quantity withdrawn, mixed, compared and returned to the bulk interferes with the titration curve to a negligible extent even in such slightly buffered fluids as Sunflower sap. The quinhydrone electrode if available, is even more convenient since the titration can be carried out actually in the electrode vessel, and the numerous points obtained give a smoother curve.

Another advantage of the capillator method is that it can be used freely with self-coloured fluids, provided that the colouring is not too dense. The usual tints disappear almost entirely when the fluids are viewed through a layer the depth of which is the inner diameter of a capillary tube.

Still another advantage of this method is the small quantity of fluid required for each determination. In dealing with plant fluids which are difficult to obtain in quantity, e.g. sap from specific tissues or from individual seedlings, this capillator method makes possible pH determinations which could not be obtained by comparator methods.

3. ERRORS

The capillator method is liable to all the specific errors of indicators as given in Chapter V. As already stated the self-colour error is to a very large extent avoided. More caution, however, is necessary when dealing with very dilute solutions.

(a) *Very dilute solutions.* — This possible error which is discussed in the previous chapter becomes more important when equal volumes of fluid and of indicator are used. The relatively small volume of indicator solution in the comparator methods becomes a relatively large volume in the capillator methods, so that with very slightly buffered solutions the indicator solution may buffer to such an extent that the virage does not change when a mixture is made with an equal volume of the fluid under investigation.

(b) *The alcohol error.* — As the red (acid) forms of methyl red and diethyl red are stable only in alcoholic solutions this requires special attention. These indicators are usually supplied, if in solution, in an alcoholic medium. Unfortunately the alcohol errors of these two indicators have not yet been determined but the magnitude of the correction to be applied to these colorimetric determinations in alcoholic media of 10 to 20 per cent. strength may be judged from the following corrections given by KOLTHOFF (p. 184); — thymol blue in 20% + 0·02; B.P.B. in 10% + 0·23; methyl orange in 10% — 0·10, in 20% —0·20. With the comparator methods the alcoholic indicator is so diluted that this error is negligible, except with industrial alcoholic fluids such as wines; but with the capillator method the indicator fluid is diluted with only an equal volume of aqueous fluid so that this error *may* amount to 0·1 or 0·2 with the methyl red type of indicator.

Clearly it depends upon the material used as to whether the advantages of the capillator method outweigh the increased liability to the errors with unbuffered or alcoholic fluids.

CHAPTER VII

SPECIAL INDICATOR METHODS

1. PRINCIPLES. 2. ERRORS

1. PRINCIPLES

The standard buffer solutions with known concentrations of indicators viewed through the same thickness of layer have been replaced in some cases by the coloured chart given by Clark (1922), in others by tintometer glasses (Sondén 1921), in others by colorimeters of various kinds, e. g. Kober (Dugger and Dodge) and Duboscq (Atkins), and in others by coloured inorganic solutions (Kolthoff 1922).

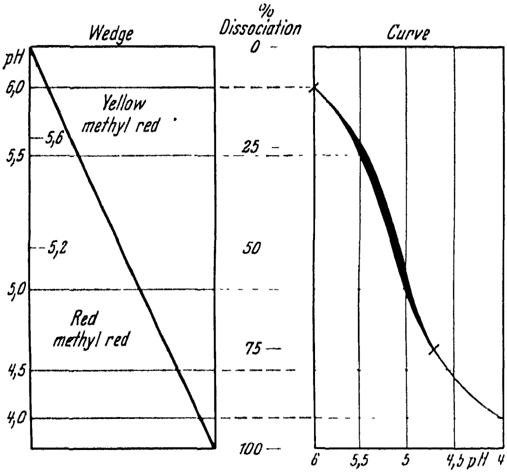

Fig. 3. Methyl Red colour wedge and dissociation curve.

Double wedges have been used by a number of investigators, as mentioned in Chapter V, and this method has been adapted to microscopic investigation by Vlès (1926). (See also Pfeiffer 1927).

The principle is illustrated in fig. 3.

Kolthoff and others have worked on indicator papers like litmus paper, but the use of these is limited to certain indicators and mainly to solutions where the buffering is strong enough to overcome the buffering effects of the paper used. Kolthoff (1926 p. 230) gives a list of indicator papers of practical usefulness for approximate measurements, together with their sensitiveness to HCl and NaOH. Lacmoid paper, which was tested by Hempel (1917) and used by Haas (1919), is rejected by Kolthoff because "the colour change was not sharp enough".

According to Clark (1928) the indicator paper is either sized and exerts a strong buffer action or it is unsized and exerts a capillary effect giving "rings of different composition". These rings are easily demonstrated by dropping indicator solutions on to filter paper.

In dealing with plant cells and tissues where the buffering is often not really strong, the use of indicator papers is, therefore, not to be recommended.

Quite a number of other methods have been used by biologists, such as silk threads coloured with indicator solution (Rebello 1922); drops of indicator applied directly to the surface (Gräff 1924); the same allowed to dry (Memmesheimer 1924); sections or cells immersed in indicator solutions and compared with tints *in vitro* Bethe 1914—22; Hurwitz 1915; Rohde 1917, 1920; Barnett and Chapman 1918; Atkins 1922; Lewis and Felton 1922; Pearsall 1923; Balint 1924; Schade 1924; Mevius 1924; Gicklhorn and Keller 1925; Pfeiffer (N. P.) 1925; Küster 1926—27; Smith 1926 and others. Sacharowa (1925) used sections immersed in very dilute indicator solutions and compared the tints with those given by phosphate mixtures in capillary tubes 6 cms by 1 mm, using an Abbé projection apparatus for bringing the two virages into juxta-position. Pfeiffer (Z. M. 1925) used the section immersion method with a modification of Gillespie's method of comparing the tints of indicators in the absence of standard buffer solutions.

A valuable method of restricted application consists of the use of natural indicators as they occur in the cells of flowers and other parts of the plant. This was first used by Schwarz (1892) and later by **Willstätter** (1914), **Haas** (1916), Crozier (1919), Atkins (1922), **Smith** (1923), **Brooks** (1926) etc. These natural indicators have been used *in vitro*, e. g. litmus; blueberry juice

(WATSON 1913); red cabbage (WALBUM 1913 and McCLENDON 1914).

Indicators have been introduced into the animal cell — (1) in solution by careful rupture (micro-écrasement) of the membrane (VLÈS 1924); (2) in solid granules (SCHMIDTMANN 1924—25); and (3) in solution by micro-injection (KITE, CHAMBERS, PETERFI and NEEDHAM, cited by REISS 1926; and others, see PFEIFFER 1927 p. 447) but such methods have not yet been applied to plants. The micro-injection method may be of considerable value provided the technique and interpretation of the results follow those of the Range Indicator Method described in the next chapter.

Other micro-methods include that given by WAGNER (1916) who compared drops of plant juice plus indicator (lakmosol) with drops of buffer solution plus indicator, the plant juice being extracted by means of a fine glass pipette. He claimed an accuracy of ·025 for his pH figures but it will be obvious that the inherent experimental error in all indicator methods applied to cells and tissues is more than this. Even for expressed juices the 'dilute solution' and 'alcohol' errors may be both of greater magnitude than ·025. FELTON (1921) developed this method of dealing with small quantities of fluid, using white 'opal' glass as a "spotplate" and direct vision for comparison (cp. SACHAROWA's capillary tube method). A similar method was used by BROWN (1923) in which the spot-plate had depressions for holding the mixed fluids. This apparatus is available from the LA MOTTE Co. (cited by CLARK). The drop method or some other modified micro-method for small quantities of sap has been used by CROZIER (1919), ATKINS (1922), SACHAROWA (1925), BROOKS (1926), MEVIUS (1926), DOYLE and CLINCH (1926, 1928). Other investigators along similar lines include MICHAELIS and KRAMSZTYK (1914), SKRAUP (1916), HEMPEL (1917), CLEVENGER (1919), HAAS (1920), RONCATI and QUAGLIARIELLO (1921) and GUILLAUMIN (1922—23).

Some of these biological special methods are so liable to gross errors that no more need be said of them; but the microcolorimeter, used in the microscope as suggested by VLÈS (1926), and the observation of the absorption spectrum using two characteristic bands of the two forms of the indicator, this microspectrophotometric method might avoid many of these errors.

Attempts to apply this method to plant tissues have, however, not proved very successful. The absorption spectrum of a coloured living cell with a cellulose wall is not easy to compare with that of a fluid in a bottle. The technique may be capable of further development.

The natural indicator method is one of the most useful ways of investigating changes in acidity under conditions which are either completely natural or as nearly natural as is at present attainable. Schwarz (1892) correlated *in vitro* the gradual change from red to blue during the anthesis of flowers of *Pulmonaria*, *Anchusa* and *Lathyrus* with a decrease of acidity and its effect upon the anthocyan indicators of these flowers. Willstätter (1914) noted that the same anthocyan indicator gives the red of the rose petal at pH 5·5 and the blue of the cornflower corolla at pH 7·2. He further found that in buffered solutions the rose goes blue at pH 7·2 and the cornflower goes red at pH 5·5. Haas (1916) investigated the anthocyan indicators of a number of plants and by means of these observations obtained quite natural values for the hydrion concentration of the cell sap (see Chapter XVII). Smith (1923), Thomas (verb.) and others have used these flower indicators in researches upon the behaviour of plant cells under experimental conditions.

The section-immersion method as used by Rohde, Atkins and others is considered in the introduction to the following chapter but in spite of the errors indicated there, this method compares favourably with most of the biological special methods.

The centrifugal infiltration method (Z.I.M.), devised by Weber (1927) is simple in operation and avoids the 'shock-effects' of section methods. In this case the whole leaf, or other organ, is placed in the test-tube of a centrifuge and completely immersed in the indicator fluid to be used. Rotation at 1760 to 2600 revs. per min. is followed by a penetration of the vital stains or indicators. In addition to Weber, Scarth (1927 p. 502) and Gicklhorn (1927 p. 7) have used this method with interesting results. Gicklhorn (Keller and Gicklhorn 1928, p. 1237) has devised a modification (V.I.M.) in which evacuation, by means of a vacuum pump, of a chamber containing the plant organ immersed in the staining fluid, takes the place of the centrifuge in the Z.I.M. Kisser (1928) discusses these methods but gives no pH data. The microscopic examination of the guard-cells of whole leaves is comparatively easy but these infiltration methods are limited in their application by the difficulties of examining the tissues of most plant organs in the whole condition. The complete displacement of the intercellular carbon dioxide may introduce a considerable error by disturbing the carbon dioxide balance of the tissues. But the errors of interpretation as indicated for other methods in the following chapter are likely to be greater than this carbon dioxide error.

2. ERRORS

Most of these micro-methods are liable to the specific errors of indicators and also the errors noted for the capillator method, e. g. dilute solution error or concentration of indicator error, alcohol error with other than aqueous solutions. The error of using methyl red and diethyl red in the stable alkaline water-soluble form, especially with relatively unbuffered sap, should be quite clear and the avoidance of the alcohol error is a matter of very careful technique (see next chapter).

When dealing with cells and tissues there may be other sources of error such as. —

Self-colour of the Cells. — Where the cells of a plant tissue already contain a pigment, indicator measurements can be applied by adding the same pigment in similar concentration to the buffered indicator solutions used for comparison, or by using colour filters of the same tint as that of the cells under consideration, when viewing the buffered indicator solutions. In either case the present writer considers that the addition of indicator solution to a cell which already contains a pigment is apt to give a colour which may be described as aleatory. Records of pH obtained by means of indicators for cells containing anthocyan pigments can be viewed only with reserve.

Concentration of the Indicator. — The concentration of the indicator naturally controls the intensity of the *tint* and may even control the *colour* in a dichromatic fluid. The concentration of the indicator must also be very low in comparison with the concentration of hydrogen ions; with comparatively strong solutions, the indicator itself acts as a buffer behaving like the salt of a weak acid and, therefore, not changing its virage to correspond with the real pH.

Localisation of the Indicator. — The indicator may be taken up by one part of the cell and not by other parts. Care is required in the attribution of the recorded pH to sap or to protoplast or to wall. Plasmolysis forms a useful method for checking this localisation in some materials. Adsorption by non-aqueous particles may interfere but this appears to be a rare case, as most of the intracellular granules which fix the colour are permeated with water. The indicator is then in aqueous solution, and behaves normally. Fine particles of mastic in a solution of sodium chloride may show yellow with bromo-thymol blue, while the circumambient fluid remains blue as stated by REISS (1926 p. 63). Clove oil, however, takes up methyl red and diethyl red in either the red or the yellow form according to the acidity or alkalinity of the aqueous fluid with which the oil is shaken.

The dielectric constant of the protoplasm. — This, as indicated by GICKLHORN (1926) and PFEIFFER (1927) (cf. KELLER 1928), is important in dealing with the few records available for the pH of cytoplasm itself; but the dielectric constant would not appear to be a serious source of error in dealing with sap or expressed juices.

Chemical change of the indicator. — Reduction, the most likely source of an error of this kind, does not interfere with the usual indicators to any considerable extent.

Solubility of the indicators. — This factor enters in one or other form into several of the other errors; but one aspect requires special mention. The acid (red) forms of methyl red and diethyl red crystallise rapidly from aqueous solutions, so that when these indicators are used on very acid material, e. g. sections of *Pelargonium*, the red form of the indicator tends to crystallise out after the indicator has penetrated to the very acid sap.

When all these possible sources of error are considered it becomes clear, firstly, that a method which avoids as many of them as possible is very desirable, and secondly that both technique and the interpretation of observations should be the subjects of careful criticism.

CHAPTER VIII

THE RANGE INDICATOR METHOD

1. GENERAL. 2. THE R.I.M. INDICATORS. 3. TECHNIQUE. 4. THE EFFECTS OF ETHYL ALCOHOL ON PLANT TISSUES. 5. DIFFUSION OF ELECTROLYTES. 6. BEHAVIOUR OF INDICATORS. 7. SPECIAL PRECAUTIONS. 8. R.I.M. AND OTHER METHODS. 9. R.I.M. ERRORS

1. GENERAL

When the facts given in the previous chapters are considered, it is clear that our knowledge of reaction or hydrion concentration in plants has been confined mainly to the results of determinations of the hydrion concentration of liquids which have been expressed from various parts of the plant and examined either by means of indicators or by electrometric methods. In addition to these determinations we have the observations by SCHWARZ (1892), HAAS (1916) and others, where the coloured substances occurring naturally in the cell were taken as indicators of hydrion concentration. ROHDE (1917) seems to have been the first to publish a record of the use of indicators applied externally to plant sections in order to determine the pH of the tissues. ATKINS (1922) followed this author in the use of the ordinary synthetic indicators in an attempt to determine the reaction of living tissues. The methods of ROHDE and ATKINS involve a comparison of the tints yielded, thus with the latter, "Using methyl red, the sclerenchyma and bast fibres give a salmon pink to pink colour, corresponding to pH 5·4 to 5·2; the wood appears a faint pink, not more acid than pH 5·4 to 5·6 and the medullary rays are yellow. The upper limits of acidity are therefore fixed. It remains to determine the acidity of the portions appearing uniformly yellow of the same tint with di-ethyl red. With phenol red the full yellow appeared, showing an acidity of pH 6·6 or more. With brom thymol blue a yellow with a green tinge was seen, matching the colour with pH 6·2". In order to avoid an error here due to a green tinge in the tissue a drop method with expressed sap was used. This method applied to fresh sections of *Salvia verbenacea* gave pH 5·2—5·4 for the sclerenchyma and bast fibres, pH 5·4—5·6 for the wood walls and pH 6·0 for medullary rays, medullary and cortical parenchyma. ROHDE uses "rosen rot, rot orange, orange gelb" and "Kirsch rot, rot orange, gelb orange, gelb" for methyl orange and methyl red respectively.

When one considers — 1. that a carefully prepared standard indicator colour is generally regarded as necessary for accuracy even to ·2 in pH values; 2. that the concentration of the indicator in the solution to be tested must not differ materially from that in the standard solution; and 3. that both solutions are usually placed in test-tubes of standard size and viewed in a comparator which permits a view only through the whole thickness of the fluid, one is rather surprised at the publication of these methods of using indicators on sections. The possible errors involved are numerous, and include 1. variation in depth of colour due to variation in the thickness of the sections, 2. variation in depth and/or quality of tint from tissue to tissue due to variation in the rate of penetration and/or adsorption of the colouring fluid, 3. variation in the quality of tint due to sap which has escaped from cut cells, 4. the 'protein' error, 5. the 'salt' error and 6. the 'lipoid' error.

The first two sources of error are so obvious to a critical investigator that CLARK (1922 p. 118), referring to "the use of unequal depths of solutions through which the colours are viewed" writes that "There are errors of technique . . . . which may be passed over with only a word of reminder". He also refers to certain optical effects (ibid. p. 65), e.g. where a dichromatic purple such as that of brom phenol blue may appear red or blue according to the thickness or thinness of the layer of fluid traversed by the light examined.

With regard to the second source of error we have in fact found tissues which, although giving a stronger red with di-ethyl red than surrounding tissues, give a yellow with methyl red while the surrounding tissues give a red with methyl red, and similar results have been obtained in other cases with other indicators. The depth of the tint observed is, therefore, no certain guide to the exact hydrion concentration of a tissue.

The third source of error can be avoided by suitable washing. The fourth or 'protein' error has its source in the removal by adsorption or otherwise, of the indicator from the field of action and must be considered in the case of the living cell. The fifth or 'salt' error has been discussed above (p. 29) and is covered by the admitted experimental error of all indicator work on plant material. The sixth or 'lipoid' error, with true lipoids, is covered for DER and MR by the use of colours only and the elimination of 'orange' and all other 'neutral' tints from the *significant* indications given by the R.I.M. method. The reds obtained with methyl red and cutin, suberin, lignin etc., would, therefore, appear to be true 'virages'.

Notwithstanding the possible sources of error in this method of determining the hydrion concentration of plant tissues, such a method is surely more reasonable and more likely to bring out a closer approximation to the truth concerning the significant variations in reaction in plants than are the electrometric methods so commonly used. It seems impossible at the present stage to apply electrometric methods to the interior of ordinary living plant cells. The nearest approach to an unmixed sap used for such determinations is that of *Valonia* used by CROZIER (1919) and TAYLOR and WHITAKER (1927). But considering the comparatively extensive variation in the pH of neighbouring cells, the determination of the pH of a liquid, which is a crude mixture of cell wall fragments, cytoplasm and vacuolar sap with most of their inclusions, does not seem capable of providing us with very satisfactory data upon which to base conclusions concerning the rôle of hydrion concentration in the life of the plant. The data obtained by CROZIER concerning the reaction of liberated vacuolar sap of *Valonia* may be more reliable and more significant, but even in that case the very process of liberation may have involved significant changes in the hydrion concentration of the sap. For example, in the case of a very slightly buffered phosphate solution the carbon dioxide content may be the factor governing the pH of the sap and wounding the cell may profoundly alter the carbon dioxide content (cp. Chapters XIV, XVII and LILLIE, 1909); allowing the carbon dioxide of the sap to attain an equilibrium with the air will certainly alter that factor.

GICKLHORN and KELLER (1926, Tabelle 4) applied fifteen different indicators and used the R. I. M. intrepretation in an investigation of *Daphnia*.

The usual methods, involving the expression of the sap, seem to the writer to have somewhat the same significance as would determinations of the pH of an average chemical laboratory by a process which involved as its first step the crushing and mixing of all the receptacles and their contents. Quite apart from the inevitable interactions, the resulting data, while more or less true as a kind of average, would furnish no very obvious clues as to the processes included in the normal activities of the laboratory. Nevertheless, many of those who use these methods work their data out to the second decimal place and some even claim a significance for variations in the last figure of such data. It should be clear, from the facts already known concerning the variation of pH from tissue to tissue, that the relative development of the various tissues must be studied and taken into account, before any reliance at all can be placed upon small variations in the hydrion concentration of a mixed liquid derived from a heterogeneous mass of tissues.

2. THE R.I.M. INDICATORS

Any indicator method of determining hydrion concentration which depends upon a finely developed colour-sense does not seem to be capable of general application, except in the hands of a trained colour specialist.

The Range Indicator Method described below[1]) depends upon the fact that in a two-colour indicator series one colour is what may be called a dominant. Thus red or blue is dominant over the yellows given by most of these indicators, i. e. on dilution or thinning of the layer viewed, without any change in pH, the pink or orange or green tints retain the red or blue element of the tint more conspicuously than the yellow element. The actual tint may theoretically and to the trained colour specialist remain the same, but the apparent tint of red diluted becomes pink, pink diluted becomes paler pink, orange diluted becomes doubtful whereas pinkish orange becomes pink, and similarly with blue to pale blue, green to pale green, yellowish green to green; whereas yellow diluted becomes apparently colourless at a much earlier stage of dilution or thinning.

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[1]) See SMALL 1926, also REA and SMALL 1926–27, MARTIN 1926–27. INGOLD and SMALL 1928.

In the range of tints given by any one of the indicators used there is a point where the yellow is definitely yellow and another where the colour becomes definitely dominated by the other element, either red or blue, i. e. the dichromatic tint becomes definitely pink or green. Between these points is usually a range of 0·4 in the pH; and it is this part of the range and this part only which we use to obtain our results. The "Capillator" colours give some idea of the range to be used, but the indicators there are concentrated.

The following table shows the scheme used with given indicators, and the method has been extended occasionally to other indicators.

Table I

Indicator	Ab-brtn.	Alk. Colour Range	pH	Acid. Colour Range	pH
Bromo-cresol purple . .	BCP	pale blue to deep purple	> 6·2	yellow	< 5·9
Di-ethyl red	DER	yellow	> 5·9	pale pink to deep red	5·6
Methyl red	MR	yellow	> 5·6	pale pink to deep red	< 5·2
Benzene-azo-α-naphthylamine	BAN	yellow	> 4·8	pale pink to deep red	4·4
Bromo-cresol green . .	BCG	pale green to deep blue	> 4·4	yellow	4·0
Bromo-phenol blue . .	BPB	pale green to deep blue	> 4·0	yellow	< 3·4
Extra Indicators					
Bromo-thymol blue . .	BTB	green to blue	> 6·4	yellow	< 6·2
Phenol red	PR	pink to red	> 7·0	yellow	< 6·8

In considering this table it should be noted, firstly that no degree of dilution will make yellow appear pink or green, and secondly that the pale blues, greens or pinks do not appear yellow on dilution. The only case where any difficulty has arisen in the interpretation of the results is with BAN. Here the yellow is not the same distinctive colour as in the other series, but with strict attention to the quality of the yellow as seen in the test-tube alkaline range there has been no further trouble in the classification of the colours found. Bromo-thymol blue has been found very useful and may replace BCP with advantage with some materials.

3. TECHNIQUE

Hand sections of fresh material are cut of such thickness that the section always has the parenchymatous layer in some part just one cell thick. These sections are carefully washed with neutral water or fresh conductivity water to remove the liberated contents of broken cells. This neutral water is prepared by nearly filling a bottle or washing flask with freshly distilled water which has invariably been found to be acid to phenol red; introducing a few drops of that indicator which has its point of least colour at pH 7; and then adding enough of the normally slightly alkaline tap water of Belfast, until when mixed the water is practically colourless. A distinct although very pale yellow tint is corrected by the addition of more tap water, while a distinct pale pink tint is corrected by the admixture of more distilled water. The presence of the minute quantity of phenol red does not interfere in any way with subsequent operations, and in the bottle it acts as a constant check on the continued neutrality of the washing water. The latter is a very necessary precaution as will be seen later.

Three washed sections from the same part of the plant are then placed in each watch-glass of aqueous (or *diluted*[1] alcoholic) indicator solution, left for 30 to 60 minutes[2]) or longer, washed again with neutral water, examined under the microscope, and the colours are then recorded. Control sections in each case are used to check the natural colouration which may interfere with the tests, especially in the epidermis. The natural colour is sometimes pink or red and the results with MR and DER are therefore subject to special interpretation.

By carefully controlled experiment it has been found that although the colour with indicators deepens during the night it seldom changes from yellow to pink or green or vice versa. The tint varies but the kind of colour usually remains the same, exceptions have been found to this in the sunflower and the potato. The indicator solutions used are those prepared by the British Drug Houses as ready for use, and the usefulness of our method

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1) See below p. 53 for the importance of this dilution.

2) Immediate examination is advisable as a check, see below p. 54.

is confirmed by the fact that after an experience of four years we were able to detect alkali coming from the bottles in one batch of solutions. The solutions were afterwards obtained in amber glass bottles which do not yield alkali to the solutions. The composition of these indicator solutions is given as follows ·

Bromo-thymol blue	BTB	0·04 % mono Na salt in 20 % alcohol
Bromo-cresol purple	BCP	0·04 % mono Na salt in 20 % alcohol
Di-ethyl red	DER	0·02 % in 60 % alcohol
Methyl red	MR	0·02 % in 60 % alcohol
Benzene azo-α-naphthylamine	BAN	0·01 % hydrochloride in 30 % alcohol
Bromo-cresol green	BCG	0·04 % mono Na salt in 20 % alcohol
Bromo-phenol blue	BPB	0·04 % mono Na salt in 20 % alcohol.

All these, except BAN, can also be obtained in aqueous solution.

It will be noted that the indicators are in alcoholic solution. By carefully controlled experiments with aqueous solutions made up from dry B. D. H. indicators Miss S. H. Martin was able to demonstrate conclusively that there was usually no difference between the kind of colour given by sections with the diluted standard solutions1) and that given with fresh aqueous solutions of the same indicators. Aqueous methyl red (B. D. H.) used for some time gave no difference in the colour but gave paler tints of the same kind of colour. Aqueous diethyl red may give more brilliant colours than the alcoholic solution.

In some cases the indicator was taken up by the cells to such a small extent that certain tissues appeared colourless. In many such experiments the sections were mounted in a hanging drop in a gas chamber and when carbon dioxide was passed through the chamber a reddish tint with methyl red or di-ethyl red resulted, showing that the yellow of the alkaline range of these two indicators when very dilute may appear colourless in the sections under the microscope. Red being a more dominant colour shows up when the pH is changed to the acid range of the indicator. Similar experiments with the yellow of the acid range

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1) For the effect of alcohol see below, section 4.

of bromo-cresol purple gave similar results with ammonia vapour or, when the pH appeared to be about 5·9, with carbon dioxide; at the latter point neither yellow nor purple is very distinct.

The indicators given above enable us to distinguish the following ranges of reaction or pH.

Colours and Indicators	pH Range	Symbols for pH Ranges	Notes
blue to purple BCP	> 6·2	A	
yellow BCP, yellow DER	< 5·9 > 5·9	a	approx. 5·9 1)
yellow BCP, indefinite DER, yellow MR	< 5·8 > 5·6	b	
red DER, yellow MR.	< 5·6 > 5·6	c	approx. 5·6
red DER, indefinite MR, yellow BAN	< 5·6 > 4·8	d	
red MR, yellow BAN.	< 5·2 > 4·8	e	
red MR, indet BAN, green to blue BCG	< 5·2 > 4·4	f	
red BAN, green BCG	< 4·4 > 4·4	g	approx. 4·4
red BAN, indet BCG, green to blue BPB	< 4·4 > 4·0	h	
yellow BCG, green to blue BPB.	< 4·0 > 4·0	i	approx. 4·0
yellow BPB	< 3·4	k	
Wider Ranges			
Indefinite			
yellow BCP, green to blue BPB.	< 5·8 > 4·0	X	— (a — h)
red DER, green to blue BPB . .	< 5·6 > 4·0	Y	— (d — h)
Acid range of first series			
red MR, green to blue BPB . .	5·2 4·0	Z	(e — h)
Higher Ranges			
yellow BTB, indet BCP, yellow DER	< 6·2 > 5·9	B	lower than the indet. range A
yellow BTB, blue to purple BCP	< 6·2 > 6·2	C	approx. 6·2
yellow PR, indet BTB, blue to purple BCP	< 6·8 > 6·2	D	
yellow PR, green to blue BTB .	< 6·8 > 6·4	E	

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1) The yellow with BCP is stronger than the indefinite purple about 6·0 — 5·8.

These symbols have been used throughout the analyses of the reactions of tissues as it is much easier to follow in this way the changes in reaction of the various tissues and from tissue to tissue in a section.

It should be noted that in addition to the indicators determining each range there are others; the colours given by these others act as an additional check on the values obtained for the hydrion concentration.

The ranges indicated by X and Y are taken as too wide to be useful and have been eliminated from the records of results which follow in Part III. Such ranges occur in practice because BAN and BCG were not used at first, and also because when used later they and also other indicators sometimes failed to give a definite colouration.

The colour resulting from each experiment in each tissue is noted and from these colours the pH range can readily be determined and translated into a ledger as one or other of the symbols (letters).

From the above account it will be seen that, when a definite kind of colour rather than a tint is taken, the results with fresh sections and a series of indicators can be quite definite and precise within the limits imposed by the use of *ranges* of pH rather than guesses at more exact figures. The use of a series of indicators in this way may reduce the width of the range indicated, e. g. *b* or 5·9 — 5·6, or it may bring the range within narrower limits, e. g. *c* or 5·6 — 5·6. The other indicators employed in each case not only check higher or lower ranges, but may also differentiate other tissues of the same section within similar narrow limits.

The Range Indicator Method (R. I. M.) as described above, compared with most other methods of using indicators for the determination of the hydrogen ion concentration, differs from these in the elimination of the central portion of the so-called "useful range" of each indicator. The essential features of the R. I. M. are (1) the use of indicators only when they show definite colours, i. e. red, yellow, green, or blue, and (2) the elimination of intermediate tints such as orange, pinkish orange, yellowish green, etc. This procedure results in the determination of ranges within which lie the actual concentrations of hydrogen ions. These determinations when used for plant tissues have a comfortable certainty which is lacking in the data supplied by "tint" methods; and the use of a series of overlapping indicators results in a reduction of the range of pH indicated to something closely approaching that obtained by most "tint" methods.

The R. I. M. has been applied, with results which are nearly always consistent, in an extensive investigation of pH conditions in plant tissues, see Part III of this volume. Nevertheless the differentiations obtained later in the investigation were felt to be not quite so detailed as seemed probable. This matter was brought into prominence when C. T. INGOLD made some preliminary observations upon a coenocytic fungus, and found that the hyphae apparently took the colour of the circumambient fluid. A high degree of permeability seemed to be indicated; diffusion was a matter of minutes. A possible cause for this phenomenon was suggested by the alcoholic medium used in the standard B. D. H. indicator solutions. The following sections[1]) deal with experiments concerning these alcoholic media and emphasis is laid upon certain points of technique in consequence of the facts observed.

4. THE EFFECTS OF ETHYL ALCOHOL ON PLANT TISSUES

A. DIFFUSION OF ANTHOCYAN

The escape of the red pigment from the cells of beetroot is often taken as an indication of death. Using this escape of the red anthocyan as an indicator of death the time for death to take place in various concentrations of alcohol was determined.

Discs of uniform thickness (2 mms. thick) were prepared from cylinders of beetroot tissue cut out with a cork borer. These discs were then washed in tap water until the water bathing them no longer showed a pinkish tinge.

An arbitrary colour standard was made by boiling one disc in 20 ccs. of water. A series of specimen tubes were prepared each containing 20 ccs. of alcohol of various strengths (0, 5, 10, 15, 20, 25, 30 and 50 %). This series was made in triplicate. Three of the washed discs were then placed in each tube. For each tube the time was taken from the moment of immersion of the discs to the time when the solution in the tube became of the same depth of colour as the standard. The results are given below.

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[1]) See INGOLD and SMALL (1928).

Strength of alcohol in volume per cent.		0	5	10	15	20	25	30	50
Time (in minutes) to reach standard	I.	over 1500			353	128	75	45	30
	II.	over 1500			349	109	69	39	26
	III.	over 1500			—	137	72	52	27
Average		over 1500			351	124	72	45	27

B. GERMINATION OF SEEDS

The action of alcohol on the germination of seeds was also studied. Small muslin bags each containing 50 dry mustard seeds were prepared. Five of these bags were immersed in each of a series of alcohols ranging from 5% to 50%. At intervals a bag was withdrawn from each strength. The seeds were washed in water for several hours to get rid of the alcohol. They were then planted. The number that germinated ten days after sowing was recorded. The results are given below.

		Time of immersion in hours					
		½ hr	1 hr	2 hrs	4 hrs	8 hrs	
Alcohol strength	5% . .	50	47	47	47	45	No. of seeds germinating
	10% . .	48	50	49	45	40	
	15% . .	44	48	50	47	10	
	20% . .	49	49	47	18	0	
	25% . .	47	49	45	0	0	
	30% . .	49	49	17	0	0	
	50% . .	50	46	48	2	0	

The figures give the number of seeds that germinated, therefore the percentage germination can be obtained by doubling each.

C. PLASMOLYSIS OF EPIDERMAL CELLS

Strips of the lower epidermis of Broad Bean leaf were immersed in various concentrations of ethyl alcohol. Samples were withdrawn at intervals and tested in a hypertonic solution of cane sugar for plasmolysis. They were then irrigated with water to test for deplasmolysis. Where any cells of a strip could be plasmolysed and then deplasmolysed it was counted as living. Where there were no such cells the strip was counted as dead. The results of a typical experiment are given below.

% Alcohol	50	30	25	20	15	10	5
Time to kill (minutes)	<15	<15	<15	<15	45/70	>360	>360

D. LOSS OF DIFFERENTIAL TURGIDITY IN *TARAXACUM* SCAPE

This method of investigation depends on the fact that when a living portion of dandelion scape is split longitudinally by two cuts at right angles to one another, the four free ends thus formed bend outwards, on account of the different tension between the epidermis and the tissue inside the epidermis.

In this experiment a series of alcohol strengths were prepared. Ten portions of *Taraxacum* scape were placed in each. At intervals a portion was withdrawn and tested for tissue tension, placing in water so that the cells might exert their full tension. Death was reckoned to have taken place when all ten pieces in a particular strength no longer showed the tissue tension action. The results of an experiment are given below.

% Alcohol	50	30	20	15	10	5
Time for loss of diff. turgidity	< 15 mins	15/30 mins	120/190 mins	>24 hrs	>24 hrs	>24 hrs

E. CONCLUSIONS

From these experiments it appears that, in the four cases considered, immersion in 20% ethyl alcohol for four hours or more results in the death of the cells. There is, however, a considerable difference in the effects of 15% alcohol and a very large difference in the effects of 10% alcohol in each case. The lethal period in the case of beetroot rises from two hours in 20% alcohol to about six hours in 15% and to over twenty-five hours in 10%. Similarly the percentage germination of mustard seeds after eight hours immersion rises from zero in 20% alcohol to 20 in 15% alcohol and to 80 in 10% alcohol. The lethal period for epidermal cells of broad bean leaf rises from less than 15 minutes in 20% to about one hour in 15% and to over six hours in 10% alcohol. The lethal period of immersion as indicated by loss of differential turgidity in dandelion scape rises from half an hour in 30% alcohol to between two and three hours in 20% alcohol and to over 24 hours in 15 to 10% alcohol.

Considering these data, the original dilution of the standard indicator solutions with an equal volume of distilled water clearly had a much greater effect than was considered possible. The use of the stronger alcoholic solutions should be combined with immediate observations or the use of a longer period of immersion should be combined with dilution of the alcoholic strengths of the solutions used.

The question then arises as to the extent to which this death of the cells in the stronger alcohol solutions affects the hydrion concentration as indicated by the R. I. M.

The many times repeated tests had shown that there were no colour differences between alcoholic and aqueous indicators, and that there were no colour differences between periods of immersion of one hour to twenty-four hours. Examination of the data given above shows that with the exception of the bean epidermis, the lethal period of immersion in 20 % alcohol appears to be greater than one hour. It, therefore, appears reasonably certain that the observations made quickly are, in most cases, made upon living cells, while the later observations are made upon dead cells. The comparative tests indicate that the later observations can as a rule be taken as giving the initial pH of the living cells when the R. I. M. is used. With „tint" indicator methods the results would be too complicated by this factor to be at all reliable.

On the other hand there did seem a possibility that diffusion of electrolytes following the death of the cells might in some cases result in a mixing of the saps from various tissues with a consequent loss of apparent differentiation in the pH of these tissues. This possibility was, therefore, investigated.

5. DIFFUSION OF ELECTROLYTES

The action of ethyl alcohol in bringing about the diffusion of electrolytes from plant cells was studied using the method described by STILES (1917). In this method the outward diffusion of electrolytes is measured by determining the electrical conductivity of the solution in which the tissue is immersed.

The tissues examined were the young stem of broad bean (*Vicia faba*) and of *Pelargonium* and the hypocotyl of sunflower (*Helianthus annuus*).

The stems were cut into transverse sections 2·5 mm. thick. These were then washed for half an hour in several changes of distilled water to remove the solutes from the cut cells. The sections were then roughly dried between clean filter papers and placed in the solution. The solutions of alcohol were made up with conductivity water and absolute alcohol. Measurements of the conductivity of the solution containing the tissue were made at intervals with a KOHLRAUSCH apparatus. The temperature throughout each experiment was kept constant. The temperature from experiment to experiment varied between 11° and 16° C. In the graphs the conductivity is given in arbitrary units and the values obtained are not corrected for the presence of non-electrolytes.

In order to determine whether the final values obtained approximated to the total electrolytes in the tissue the following procedure was adopted. The tissue at the end of each experiment was boiled up in a few ccs. of the bathing fluid for 20 minutes and thoroughly crushed. The solution was then made up to its original volume with alcohol and water, bearing in mind that during the boiling all the alcohol is driven off. This procedure gives a value which is probably too high on account of the colloids set free during the boiling and crushing process.

Considering the results summarised in the graphs (figs. 4, 5, 6), it is clear that the outward diffusion of electrolytes is rapid in 20% alcohol approaching completion within three hours in the broad bean and sunflower. This is similar to the results obtained for potato tuber by STILES (1917). The sunflower and bean were chosen as species upon which the R. I. M. had been used extensively, and the investigation included the stem of Pelargonium as an example of the "acid type" distinguished by Rea and Small in their first survey of stem tissues (1926). It is, therefore, interesting and noteworthy that Pelargonium stem in 20% alcohol does not show this rapid diffusion. When 10% alcohol was used the sunflower hypocotyl showed a less rapid diffusion but after ten hours immersion the diffusion had already reached a half way position, whereas in the bean stem the diffusion in 10% alcohol was at first possibly lower than that in water. Even after twenty four hours immersion in 10% alcohol the outward diffusion in the bean was very little more than in conductivity water. The lower concentration of alcohol was not

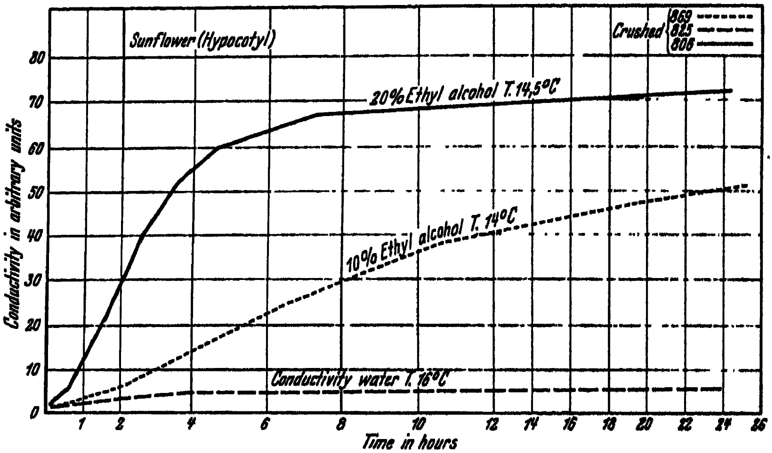

Fig. 4.

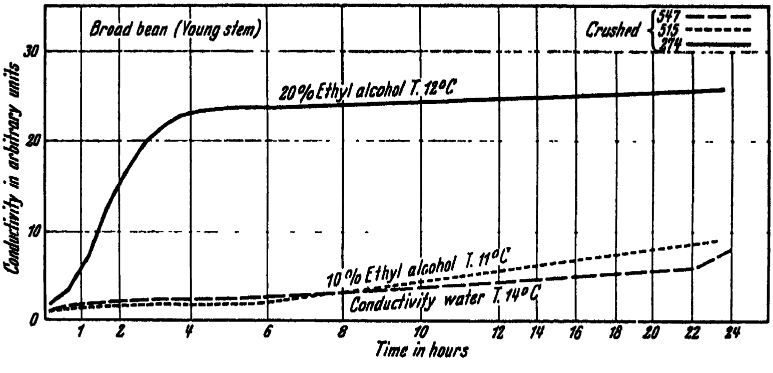

Fig. 5.

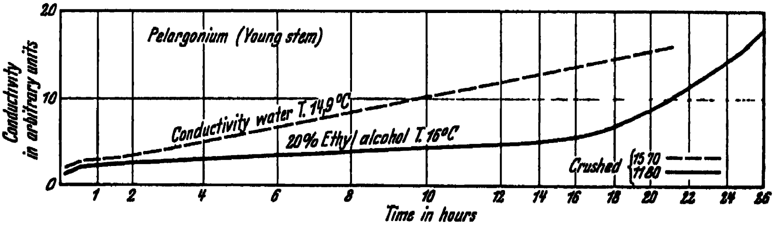

Fig. 6.

used for Pelargonium stem in view of the result obtained with 20 % alcohol.

These considerations lead to the conclusion that, in some cases at least, diffusion of electrolytes occurs with strongly alcoholic media. Such diffusion would probably not affect the indications for lignified cell walls and for some cytoplasmic contents but, where the observed pH concerns the sap or the diffusable electrolytes of the cytoplasm this diffusion might affect the results. This possibility was investigated particularly in the case of the sunflower hypocotyl.

6. BEHAVIOUR OF INDICATORS

The diffusion of electrolytes was studied in slices of tissue 2·5 mms. thick; the diffusion probably reaches its maximum more rapidly in the thinner sections used in the R.I.M. The conditions may be considered thus. — In a thin section there are some thin-walled cells with relatively acid and relatively alkaline diffusable contents; if this section be immersed in an indicator solution containing 20 % alcohol, outward diffusion of electrolytes will take place, if the tissue be of the type occurring in sunflower and bean. If diffusion be rapid the diffusable contents of the acid and of the alkaline cells will, after a time, become mixed with the indicator solution. When the section is observed after this intermixing has taken place the difference between the hydrion concentrations of the formerly acid and alkaline cells will have disappeared. It is clear, therefore, that a differentiation which was actually present may not be observed when this intermixing has taken place.

The result of these considerations has been the discovery of a group of physiologically differentiated cells in the hypocotyl of the sunflower (*Helianthus annuus*) opposite the vascular bundles. This group of cells was not differentiated as a constant phenomenon by Miss MARTIN in her survey of the sunflower tissues. She records (1926, Table IV) the pericyclic region as b (c), that is pH 5·9 — 5·6 or in exceptional cases of pH 5·2 — 4·8.

As an extreme case, but one which is of special value in differentiating these cells, di-ethyl red is taken. This is used in 60 % alcohol. The table shows the differentiation observed in sections immersed for various periods in the strongly alcoholic indicator. The colour of di-ethyl red at its neutral point is orange, but the red form of the indicator is unstable in aqueous solutions, Sunflower hypocotyl of plant at stage where three pairs of foliage leaves are expanded

Time in mins.	Di-ethyl-Red Indicator dissolved in —:	Epidermis	Sup-epidermis	Cortex	Group of cells opposite pericyclic fibres	Endodermis	Pericyclic Fibres	Phloem and Cambium	Xylem	Pith
1	60% Alcohol	pink	pink	yellow	pink	yellow	pink	yellow	pink	yellow
15	,,	pale pink	indefinite	yellow	yellow	yellow	deep pink	yellow	deep pink	yellow
45	,,	indef.	indef. to yellow	yellow	yellow	yellow	pink	yellow	pink	yellow
120	,,	indef. to yellow	indef. to yellow	yellow	yellow	yellow	pink	yellow	pink	yellow
15	Water	deep pink	pink	yellow	bright pink	yellow	bright red	yellow	bright red	yellow
45	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
120	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,

hence the strong alcohol used in the standard solution. It will be noted that the only tissues which change in actual colour are — (1) the above-mentioned groups of acid cells, (2) the epidermis, which was uniformly recorded as very acid (i. e. red with DER by MARTIN) and (3) the sub-epidermis which was found by MARTIN (1927) to vary.

The results of this investigation indicate that when indicator solutions containing more than 10 % alcohol are used without dilution with distilled water in observations by the "Range Indicator Method", outward diffusion of electrolytes may lead to a mixing of diffusable substances remotely approaching the conditions obtaining when expressed sap is used for the determination of the pH of plant juices.

Further, since the sections containing a relatively small quantity of electrolytes are immersed in a relatively large quantity of indicator solution, there may not be enough plant juice to change the colour of the indicator solution. As it has been shown (see Chap. XIX), that plant juices are in some cases only very slightly buffered, the colour observed after immersion in indicator solutions containing 20 % alcohol may depend mainly on the colour of the circumambient indicator fluid. This appeared to be the case in the cortex and pith of the broad bean and may be responsible for a certain lack of differentiation which is apparent in some of the later records of stems and leaves throughout the year. It must be remembered, however, that the results contain many instances of a differentation between thin walled cells of the cortex and those of the pith, both in the broad bean and in many other plants. Nevertheless, since the "Range Indicator Method" enables us to obtain only an approximation to the hydrogen-ion concentration of the tissues, anything which enables us either to approximate more closely to the actual pH or to differentiate the tissues to a greater extent should naturally be included in the technique of the method. We therefore put forward the following precautions and modifications which are now being used in this laboratory.

7. SPECIAL PRECAUTIONS

As a result of these investigations (1926—1928), and of the demonstration by Martin that the juices of plants may be only very slightly buffered, the following points in the technique of the R.I.M. are emphasised —

1. The use of standard alcoholic indicators only after dilution with pure distilled water to bring the alcoholic content to or below 10 %;

2. The use of specially prepared alcoholic indicators containing 10 % or less of alcohol,

3. The use where possible of aqueous indicators,

4. The reduction of the period of immersion to a minimum consistent with the obtaining of unequivocal colour indications; this period may be anything from one minute to twelve hours;

5. The use of all indicators with the indicator brought as near as possible to its neutral point, so that even a slightly buffered plant fluid may be able to throw the indicator to one side or the other of the neutral point.

In view of the conditions within many plant cells this fifth point has been found to be of special importance. The indicator solutions, as kept for use in bottles fitted with pipettes, should be corrected and maintained at the proper neutral point by the judicious use of N/20 solutions of sodium hydroxide and hydrochloric acid. Any extensive addition, even of sodium chloride, may introduce a serious "salt error", but in practice it is found to be a matter of one or two drops of either acid or alkali in 20 ccs. or more of indicator solution.

An important detail consists of the use of fresh conductivity water in preparing the neutral water described in the original technique. This water should be used when not more than two days old. The ordinary 'blow' wash-bottle should not be used, since the carbon dioxide of the breath acidifies the water quite considerably.

The placing of a coverslip over the section may induce acidification, see Chapter XIV under "The Effect of Sectioning".

Used with all due precautions, and keeping in mind that the inherent experimental error of any indicator method used on plant tissues is about ± 0·1[1]), the R. I. M. has been found a valuable method for exploring the field and preparing the way for many detailed investigations. This will be obvious after a perusal of Part III.

The Range Indicator Method is a practical process for the determination of the hydrion concentration of plant tissues, perhaps with a lesser degree of apparent accuracy but with a greater degree of certainty than is possible with the many other methods which have been reviewed in the previous chapters. It is true that what is determined by this method is the range within which the actual reaction lies, but it is also true that variations occur which bring the hydrion concentration out of one range and into another. The variations which occur under natural conditions are, in fact, so large that for the purpose of a general survey of the actual acidity of plant tissues the Range Indicator Method is distinctly advantageous.

In view of another two suggested objections to the use of indicators in the determination of hydrion concentration we

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1) Many possible errors with the same material and the hydrogen electrode, may be of ten times this magnitude, cp. TAYLOR and WHITAKER (1927) who give an average of pH 5·47, but are inclined to regard the higher value (pH 6·16) as the more reliable.

should like to point out the relation of the Range Indicator Method to these new suggestions.

Reiss (1926 p. 68) points to the possibility of the rH (oxidation reduction potential) interfering with the indications of the pH as given by dyes; but, as the Needhams' (1926 p. 291) point out, Nile blue and cresyl blue are affected by the rH of the system and "Since the former dyes command no general respect as pH indicators and are rarely used for that purpose, little trouble need be expected from this source."

The other point depends upon the relation of the protein isoelectric point to the pH of the medium and resulting adsorption of only one form of all dyes. If the adsorbing protein be in a medium which is more acid than the pH of the protein isolectric point, the protein should carry a positive charge and adsorb one form of the indicator dye; while if the pH of the medium be higher than the protein isoelectric point the protein should be electronegative and adsorb the other form of the dye.

In the first case the protein would appear to be acid with all the indicators used, and in the second it would appear to be alkaline with all the indicators used. It is clear that, if this theoretical phenomenon occurred to any appreciable extent in our work, we would get no results at all with the Range Indicator Method, except the range A (above pH 6·2) and the range k (below pH 3·4), using our normal six indicators. In actual practice the usual result is an acid indication from the upper indicators and an alkaline indication from the lower indicators, giving the limited ranges which we have recorded. The range A, above pH 6·2, has occurred only in two cases, the callus of the sieve-plates and the epidermal hairs (Martin, 1927). The latter, however, although they gave a deep blue with thymol blue indicating pH $>$ 9, were colourless with phenolphthalein indicating pH $<$ 10. Again we got the series of alkaline indications broken. The former, callus, although blue with bromo-cresol purple (pH $>$ 6·2), gave no trace of blue with bromo-thymol blue indicating a reaction at any rate less than pH 6·4. In this case also the series of alkaline indications was broken. As yet we have not investigated the reaction of tissues below pH 3·4, so that in the more numerous cases where k (pH $<$ 3·4) is recorded this objection might be upheld. It is significant, however, that tissues, usually lignified cell walls, which are recorded as k always pass through the h (pH 4·4—4·0) range in their development, where the acid series is broken at its lower end by alkaline indications (see Chap. XI). This problem awaits further investigation.

The *practical* result of a normally broken series, arranged as an upper series of acid indications and a lower series of alkaline indications, seems to us the proper and conclusive reply to these theoretical objections. Strangely assorted results would undoubtedly be obtained if the rH affected one form of the dye in one case and the other form in other dyes, especially with a series of indicators of varied chemical constitution such as we use. We do not get such assorted results, but two clear series with the break indicating the pH range in a reasonable fashion. On the other hand both objections may apply to a certain extent where the indicator method involves a comparison of *tints* as distinct from colours.

8. THE R.I.M. AND OTHER METHODS

The Range Indicator Method is definitely used, not to determine the exact pH, but to determine the range within which the exact pH must lie. The general experimental error of all indicator methods *applied to this material*, plant cells and tissues, is ± 0·1 in pH units. The R.I.M. enables the pH to be determined as lying in a range which varies from 0·4 of a pH unit to approximately (± 0·1) a definite figure on the pH scale.

The use of the R.I.M., *with all the various precautions outlined above*, in the determination of the pH of plant cells and tissues avoids entirely the large general errors of expressing mixed sap and either entirely or to a considerable degree the following errors which have been noted in previous chapters for other methods —

(a) Hydrogen Electrode Errors — due to carbon dioxide dilution, reduction, oxygen, electrode poisoning, etc.;

(b) Quinhydrone Electrode Errors — due to protein, phosphate - glucose, tannin and carbon dioxide dilution;

(c) Micro-Electrode Errors — due to the above, especially electrode poisoning, together with membrane formation and other disturbances;

(d) Comparator and Capillator Indicator Errors — due to self-colour, dilute solutions, salts, proteins, lipoids, temperature, chemical changes, etc.;

(e) Errors of Other Indicator Methods — due to adsorption, tint-comparisons involving concentration or localisation of indicators, solubility of indicators, self-colour of cells, displacement of carbon dioxide, lipoids, dielectric constant, etc.

There remains (1) the alcohol error, and (2) the toxicity of the indicator. (1) The first has been considered in detail and it would appear that alcohol in concentration below 10 % has not, as a rule, any violent action upon plant cells and tissues. The data given in the Table, p. 58, could be repeated with many species and, as there is very little *colour* change using DER in 60 % alcohol, it is reasonable to conclude that using 10 % alcoholic or aqueous indicator solutions, determinations can be made of the pH of living cells provided that the toxicity error be avoided.

(2) The toxicity of some indicators is known, but BTB, BCP, BCG, MR and DER do not appear to be rapidly toxic to plant cells. Sections can be left in aqueous indicators for hours and become distinctly coloured long before any toxic effect can be detected by means of the plasmolysis — deplasmolysis test.

This has been obvious to us in many tissues and has been definitely demonstrated by C.T. Ingold for DER and MR (in the epidermal cells of *Vicia faba*, unpublished work), and by the writer in the case of the potato. The plasmolytic test should be used as a check in detailed work on particular tissues or cells, but considering the small differences obtained, it would have been a waste of time to spend hours on such testing in the course of the general survey which has been carried out in this department. In fact, the extensive survey work was possible only because of the relative insignificance of these two errors.

9. R.I.M. ERRORS

The results obtained using the R.I.M. are liable to the following errors —

1. The determination of a pH range, rather than a point, gives the same range to two or more tissues which may be really different in their actual pH, if the differences happen to lie within the range determined. This error is minimised, but not altogether avoided, by the use of more indicators; e. g. by the breaking up of the **Z** (5·2—4·0) range into the ranges **e f g h i** by the use of B.A.N. and B.C.G.

2. The use of indicators containing more than 10 % alcohol in the earlier observations may have resulted in a mixing of saps and a consequent loss of observed differentation within a given section. This is avoided by the improved technique.
3. The use of indicators, such as B.P.B. and B.A.N., which are more rapidly toxic than the others used, may again have resulted in a mixing of the saps of killed cells with loss of differentiation within a given section of stem or other part of the plant.

These three appear to be the only serious errors to which the R.I.M., as used here, is liable. They all result in a possible loss of observed differentiation. On the other hand, the records given in Part III show quite considerable differentiation, more in fact than was observed by Rohde using a plasmolysis test for 'liveness' throughout, more in many cases than was observed by Atkins using tint comparisons.

WHERE DIFFERENTIATION IS RECORDED IT MAY BE TAKEN AS REPRESENTING A TRUE DIFFERENTIATION, BUT WHERE NO DIFFERENTIATION IS RECORDED THE RECORDS MUST BE TAKEN AS LIABLE TO ONE OR ALL OF THE THREE R.I.M. ERRORS AND, AS SUCH, SUBJECT TO REVISION AND CORRECTION AFTER FURTHER INVESTIGIATION.

CHAPTER IX

BUFFER DETERMINATIONS

„*If you can measure that of which you speak, and can express it by a number, you know something of your subject*"; Lord KELVIN (cited by CLARK, 1928 p. 119).

Substances which control or regulate the hydrion concentration, when acids or alkalies are added to solutions, are called *buffers* or *moderators*. Some substances regulate the hydrion concentration by removing one of the active ions from the sphere of action by precipitation e. g. calcium carbonate; or by adsorption e. g. charcoal, colloids; or by membrane action (cp. HOAGLAND and DAVIS 1923, and also MAC DOUGAL and MORAVEK 1927).

Theoretically equal gramme-equivalent concentrations of all monovalent acid-salt or base-salt buffer systems exert the same degree of buffer action at the point of maximum buffering, but this buffer action is exerted at different points on the pH scale. Strong acid-salt buffer systems act between pH 1 and pH 3; strong base-salt systems act between pH 11 and pH 14 (13·9); weak-acid buffer systems reach their maximum buffer action at various points between pH 3 and pH 10, while weak base-salt systems reach their maximum at various points between pH 7 and pH 11. Amphoteric proteins and other ampholytes require special consideration.

The important *individual characteristics* of a buffer system are, therefore, the point on the pH scale of the maximum buffer action and the concentration of the buffer. The first characteristic is determined by the dissociation constants, acidic Ka or basic Kb. The second characteristic may be determined experimentally in each case.

Buffer action is explained in the standard works of hydrion concentration, and here it is only necessary to indicate one or two salient points. Titration curves (figs. 7—8) are generally given to illustrate buffer action and we have the equation

$$\frac{[H^+]\times[A^-]}{[HA]} = Ka \therefore \frac{1}{[H^+]} = \frac{[A^-]}{Ka\,[HA]}.$$

Since Ka is a constant we have, in the rise of the pH [fig. 8 (a) (d)] on the addition of alkali BOH, two factors operating, firstly the decrease in $[H^+]$ through neutralisation by the OH^- ions of the added alkali BOH, and secondly an increase in the ratio $\frac{[A^-]}{[HA]}$ because of the formation of a salt B^+A^- which may dissociate much more freely than does the acid HA itself. The second factor adds dissociated A^- ions and reduces the [HA] to the same extent, thus doubly increasing the ratio $\frac{[A^-]}{[HA]}$. The relative extent to which these two factors act depends naturally upon the relative dissocations of the salt BA and the acid HA. If HA be strongly dissociated as well as BA, then the first factor predominates, as with strong acids [fig. 8 (d)], while if HA dissociates much less then does BA, as with weak acids, the second factor will predominate [fig. 8 (a)].

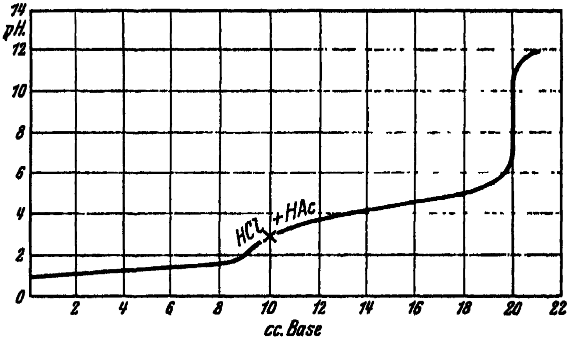

Fig. 7. Titration Curve of a mixture of 10 cc. N HCl and 10 cc. N acetic acid with N NaOH (after Michaelis).

The action of this second factor in the buffering region of weak acid-salt systems may be analysed thus —

$$\frac{[H^+]\,[A^-]}{[HA]} = Ka \therefore \frac{[A^-]}{[HA]} = \frac{Ka}{[H^+]}$$

then, Ka being a constant, the ratio $\frac{[A^-]}{[HA]}$ governs the value of $\frac{1}{[H^+]}$ and the pH. The increase in the pH is due mainly to the increase in the $[A^-]$ and decrease in $[HA]$ as explained above; but the rate of this increase in pH is governed by the *rate* of the change in the ratio $\frac{[A^-]}{[HA]}$ with the addition of alkali. The rate

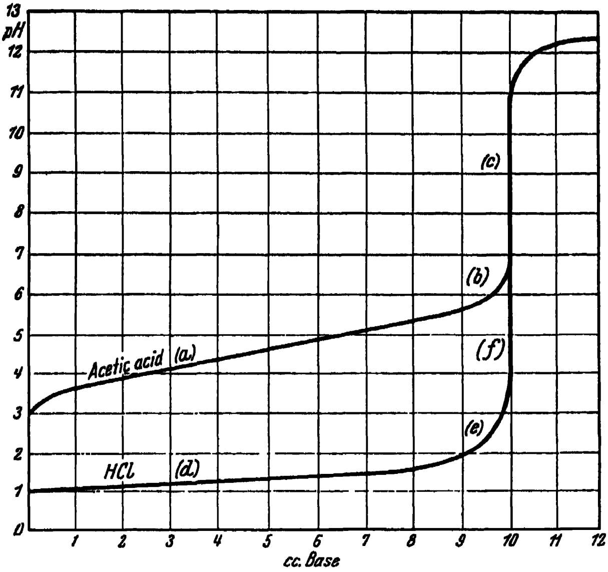

Fig. 8. Titration Curves of 100 cc. portions 0·1 N HCl and 0·1 N acetic acid with N NaOH (after MICHAELIS).

of change varies with the *difference* in the concentrations $[A^-]$ and $[HA]$, and as a simple matter of arithmetic, Ka being a constant, this rate will reach a minimum when $[A^-] = [HA]$ and increase more or less rapidly in either direction. If $[A^-] = [HA]$ then $Ka = [H^+]$, so that the increase in pH is at a minimum around the pH corresponding to Ka (i. e. pKa). This minimum increase in pH can be translated into terms of buffer action as "maximum buffer capacity" which occurs at the pH corresponding to pKa, the exponential form of the dissociation constant Ka.

Until recently (1922) only titration curves (figs. 7—8) were considered and the *degree* of buffer action was referred to as the slope of the curve. Naturally the measurement of this slope presented difficulties and the *degree* of buffer action was seldom put into figures, with a resulting obscurity for anyone who was not an expert chemist. The degree of buffer action was measured in terms of ratios of the concentrations in gm. mols. or gm. ions per litre. In 1922 however VAN SLYKE published his classic contribution to buffer action and now we can give numerical expression to these phenomena, with a surprising increase in the clarity of our conceptions of what happens in plant cells and tissues.

BUFFER INDEX

VAN SLYKE's unit of buffer action may be defined thus — a solution has a buffer index of 1·0 when the addition of one gramme-equivalent of strong acid or alkali shifts the pH of one litre of the solution through one unit.

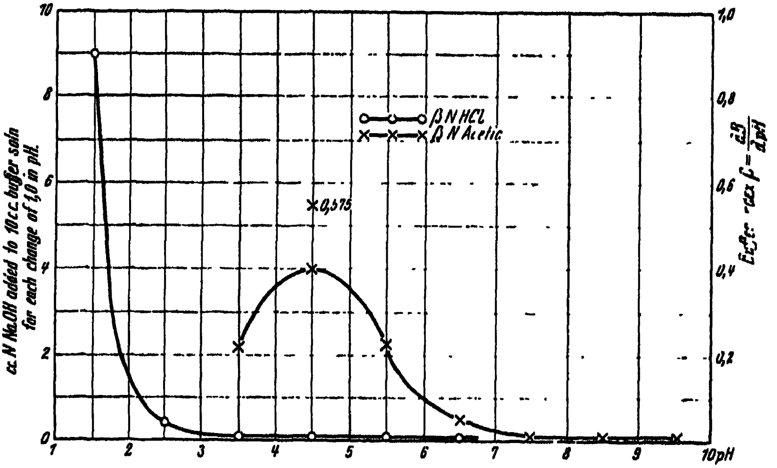

Fig. 9. · Buffer Index Curves of HCl + NaCl and HAc + NaAc.

The buffer capacity or buffer index is expressed as β and is equal to $\frac{dB}{dpH}$, where dB is the gramme-equivalent concentration *per litre* of added base and dpH is the change in the pH observed when the quantity dB is added to the solution of which the buffer index is being determined. In actual practice the formula $\frac{dB}{dpH}$ becomes —

$$\frac{\text{gm. equiv. conc. base (or acid) soln. added} \times \text{vol. base (or acid) soln. added in litres}}{\text{pH shift} \times \text{vol. buffer soln. in litres.}}$$

Comparing acetic acid with hydrochloric acid, the maximum β value for acetic acid is in the region of pH 4·73, while for hydrochloric acid it is very approximately in the region of pH 1. Remembering that pH is the reciprocal of the log [H^+], these values are approximately in the ratio of ·0000186 : ·1 = 1 : 5376. This means that, at their respective maximum buffering points, hydrochloric acid is approximately 5000 times stronger than acetic acid, cp. fig. 8. The biological significance of this fact is quite clear. The living cell cannot as a rule tolerate high degrees of acidity. The buffer action of strong acids ceases below a relatively high concentration of hydrogen ions (pH 2—3, see fig. 10) and this buffer action is, therefore, of comparatively little importance in biology. That is why 'buffers' in biology are most frequently referred to as *weak* acid-salt or *weak* base-salt systems.

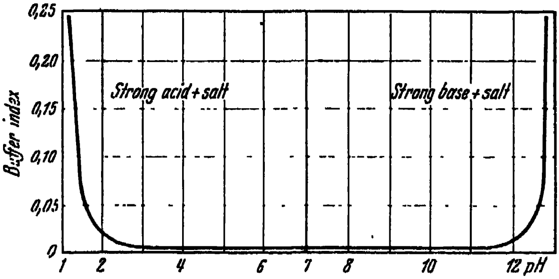

Fig. 10. Buffer Index Curves of strong acid and strong base (after KOLTHOFF)

MAXIMUM BUFFER ACTION

The mathematical argument developed by VAN SLYKE (1922) is summarised both in MISLOWITZER (1928) and CLARK (1928), so that it is here sufficient to state the end point, namely that if α be the degree of dissociation, $\frac{dpH}{d\alpha} = 2{\cdot}303\,\alpha\,(1-\alpha)$ and therefore when $\alpha = 0{\cdot}5$. $\frac{dpH}{d\alpha} = 0{\cdot}576$, the maximum buffer index of a monovalent acid in gm.-equivalent concentration.

In general, using [B] as the gm.-equivalent of base added and [S] the total acid (gm.-equiv. conc.)

$$\frac{d[B]}{dpH} = 2{\cdot}303\,\alpha\,(1-\alpha)\,[S],$$

which is at its maximum when $\alpha = 0{\cdot}5$ ($= 0{\cdot}576\,[S]$).

The maximum buffer index β is, therefore, governed as to magnitude by the gm.-equivalent concentration [S]; for ·1 molal concentration $\beta = {\cdot}0576$, for ·01 molal $\beta = {\cdot}00576$ and so on. This maximum β is further governed as to position on the pH scale by Ka or its logarithmic form pKa, since $[H^{\cdot}] = Ka$ when $\alpha = 0{\cdot}5$ (see Clark 1928 p. 17).

BUFFER INDEX CURVES

With weak acids, where the salt dissociates much more strongly than does the acid, α or the degree of dissociation is almost the same as the extent of neutralisation; the acid being regarded as the undissociated fraction and the salt as the dissociated fraction. In such cases a titration curve can be converted into a neutralisation curve as shown in fig. 9 (left co-ordinates) and this can be regraduated as a buffer index curve (fig. 9, right co-ordinates), showing in the first graduation the variation of pH with base added and in the second the variation of β with pH.

Buffer Index curves are in this way readily derived from titration curves, where the *volume of buffer solution*, as well as the base or acid added, is included in the data given. Many earlier workers have made it impossible for others to plot buffer index curves from the earlier form of titration curve by neglecting to state the volume of buffer solution used.

SIMPLE BUFFER SYSTEMS

When only one monovalent system is buffering it is comparatively easy to explain the whole action by means of a buffer index curve[1]).

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[1]) In spite of this one finds at least one investigator (Hurd-Karrer 1928) who is capable of presenting curves as $\frac{d\,pH}{dB}$, using the symbols in a new, quite empirical, sense, because it is more convenient and "just as useful for the purpose of the present investigation", apparently content

The titration curve is plotted carefully, the dB values are read off for each unit or half unit of pH 3 to 4, 4 to 5 etc., and transferred to a neutralisation curve which can be graduated as such or as a buffer index curve by a simple calculation, as shown above (p. 69). The maximum will be very approximately around the pH corresponding to the pKa of the acid[1]). Then the buffer index at its maximum multiplied by 1·736 or $\frac{1}{\cdot 576}$ will give an approximation to the gm.-equivalent concentration of the buffering system present.

If greater accuracy be desired it is necessary to plot the buffer index curve for smaller shifts in pH (·2 instead of 1·0); this gives a closer approach to the true maximum, since the buffer index varies rapidly and with a simple system quite considerably even within the range of unit pH (see fig. 8). This more accurate plotting may, however, be quite misleading when one is attempting an identification of the buffer system, since the maximal values of β become obscured in a mixture of systems unless they are at least 0·5 of a pH unit apart.

POLY-BASIC ACID-SALT SYSTEMS

These are a little more complex but the buffer action of phosphates has been shown (MARTIN, 1927, 1928; INGOLD 1929) to be of special importance in plant physiology and this type of buffer system must be considered.

As salts of a trivalent acid, phosphates occur in three forms -- K_3PO_4, K_2HPO_4 and KH_2PO_4. The titration curve of phosphoric acid with KOH (fig. 11) shows three regions in which phosphates buffer and two regions where buffer action is slight. There is first the region (*a*) in which H_3PO_4 is changing to KH_2PO_4 (pH 1·2—pH 3·5); then comes a zone (*b*) where nearly all the phosphate exists as KH_2PO_4 and small additions of alkali have a larger effect (pH 3·5—pH 5·6); a second buffering zone (*c*) occurs

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to be in isolation, and obscure if possible any comparison of the buffer indices which might have been obtained by her for the wheat plant with those obtained by others for other plants or even the same plant.

[1]) Acid buffers appear to be the chief buffers in plants; basic buffers with pKb seldom act below pH 7 except possibly in the case of proteins and amino acids.

while KH_2PO_4 is being changed by added alkali into K_2HPO_4 (pH 5·6—pH 7·8); a second zone of rapid increase of pH with small additions of alkali (*d*) with nearly all the phosphates as K_2HPO_4 (pH 7·8—pH 10·5); finally there comes a third buffering zone (*e*) where the K_2HPO_4 is being changed to K_3PO_4 (pH 11·—pH 12). Of these three buffering zones (see fig. 12) the upper one is seldom used in laboratory practice, other buffers giving better,

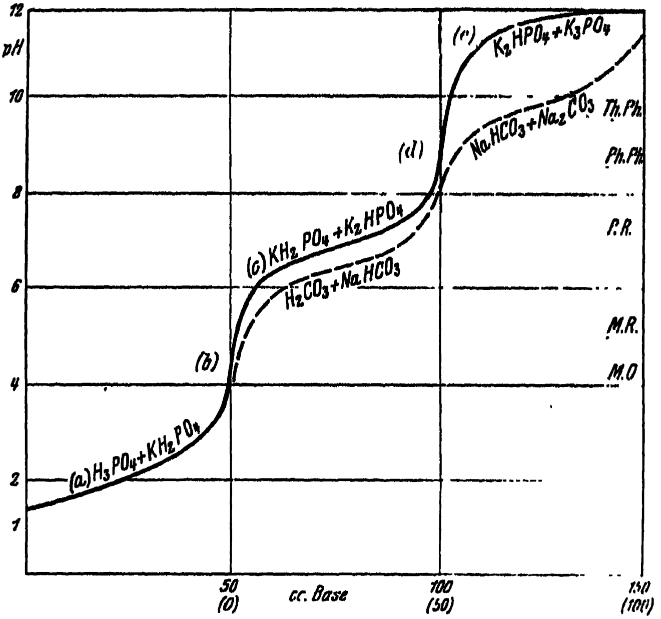

Fig. 11.

——— Titration Curve of 50 cc. M/10 H_3PO_4 with M/10 KOH. (modified after Clark.)

······ Titration Curve of Carbonic Acid. (modified after Kolthoff). Change points of indicators on right.

more readily calculated effects; the lower buffer zone is also seldom used *in vitro*, but may be of considerable importance in plants; the middle buffer zone is the most important, possibly one of the chief buffer phenomena in ordinary herbaceous plants. The dissociation exponents pKa corresponding to the three forms of acid are approximately 2·11, 7·16 and 12·66 respectively (Clark 1928 p. 678).

In general biology there is probably no more important acid than carbonic acid and the buffer action requires consideration, although many plant tissues appear to be below the zone of bicarbonate-carbonic acid buffering. The titration curve given, fig. 11, shows a general similarity to the upper two-thirds of the phosphoric acid curve. There is a lower buffering zone, where H_2CO_3 is being changed to bicarbonate (pH 5·6—pH 8·2) and this passes, with no definitely unbuffered zone, into the upper buffering zone where bicarbonate becomes carbonate (fig. 11). The dissociation exponents, pKa, for the two stages, namely, 6·33 and 10·22, indicate the maximum buffer regions around pH 6·3 and pH 10·2. Inspection of these and the second and third constants for phosphates shows that the degree of dissociation with $'H_2PO_4$ $''HPO_4$, H_2CO_3 and $'HCO_3$ is even smaller than that of acetic acid (pK 4·73).

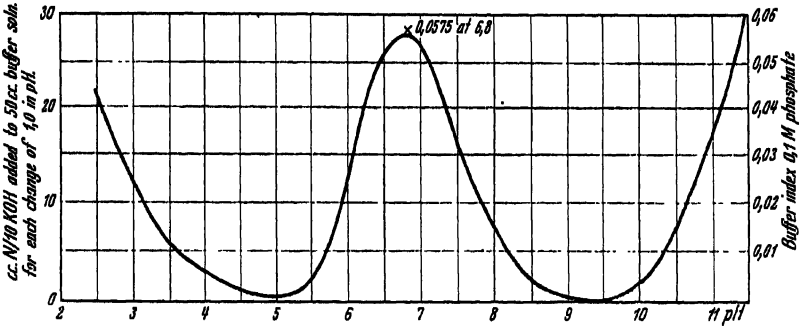

Fig 12. Buffer Index Curve of 0·1 M phosphate buffer.

The strength of buffer solutions, i. e. the buffer capacity, buffer index, or β, depends upon the gm.-equivalent concentration of the buffering substances, on the pH of the solution and that, as usual, on the temperature. Inspection of the titration curves given in figs. 7, 8, 11, will show that this buffering capacity varies with the slope of the curve. When the titration curve is vertical, buffer capacity is obviously at a minimum and it is at a maximum where the curve most nearly reaches the horizontal. This region of maximum buffer action lies around the point where the ratio of one member to the other member of the buffer system, e. g. $K_2HPO_4 : KH_2PO_4$, is 50 : 50, or taking acetic acid as an example where the acid is just half neutralised.

The buffer value throughout the given small range of pH can, therefore, be calculated in terms of the molecular concentration of the buffer provided that we know the ratio between acid and salt, or primary or secondary phosphate at each end of that range. This method is sometimes convenient and was used by **Martin** (1926, 1927) but, in plant physiology especially, it is important that comparisons be possible between one plant or stage of a plant and another. The buffer capacity should, therefore, be expressed quantitatively even in cases where the buffering substances are unknown. Such a procedure may make it possible to identify the unknown buffer system and in any case it always eliminates a number of buffers as being negligible in any particular case. It is for these reasons that light-hearted inversions or variations of presentation, e. g. **Hurd-Karrer** (1928), are to be avoided where it is at all possible.

PROTEINS AS BUFFERS

According to the new view (**Bjerrum** 1923) amino acids and proteins contain a zwitter-ion and ionise as acids or bases. From the hydrion concentration point of view proteins can be treated within limits as simple electrolytes. It should be noted, however, that the zwitter-ion $^{+}NH_3RCOO^{-}$ does not conduct electric current and that the position of the amino groups in relation to the carboxylic groups has a marked effect on the strength of the acidic characters. **Bjerrum** has also shown that the strength of the amino acids is much greater than was previously supposed. The old acid dissociation constant Ka, was about 10^{-8} to 10^{-10}, (pK 8 to 10), whereas the true dissociation exponents pK_A are 2·26 for leucin, 2·51 for tyrosin, 2·08 for asparagin, etc. (see **Clark** 1928 p. 680).

The corresponding basic dissociation exponents pK_B are for leucin 9·75, tyrosin 8·40 and asparagin 8·87. Thus asparagin acts as an acid around pH 2·08 and as a base around pH 8·87. Somewhere between these two lies a point at which acidic and basic properties are equal and nothing but zwitter-ions and neutral molecules are present. The special properties of proteins depend upon their large molecules, colloidal condition with strong adsorption capacity, strong imbibition (hydrophilic when dissociated), their amphoteric chemical behaviour and the varied positions on the pH scale of the isoelectric points of denatured proteins (cp. fig. 13).

In the recent quantitative work on buffers it was a surprise at first to find that proteins as buffers were almost negligible in expressed plant juices, but a consideration of the basis of the buffer capacity and the large molecular weight of proteins and amino-acids makes the matter quite clear. The buffer capacity is an ionic phenomenon and, therefore, the buffer capacity for equal weight in volume solutions varies *inversely* as the grammo-equivalent weights of the buffers. Taking phosphate as an example, H_3PO_4 acts at each maximal point as a monovalent

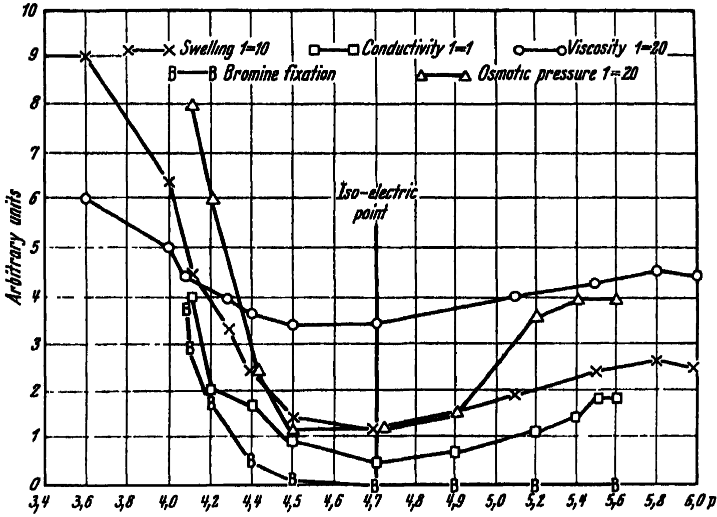

Fig. 13. Properties of gelatin in relation to pH. (modified after LOEB).

buffer system ($'H_2PO_4$ or $'HPO_4$ or $'PO_4$); the molecular weight of H_3PO_4 is 98. The molecular weight, even the valencies, of proteins are still subjects of dispute but the valency is low, one to five, and the molecular weight is high. According to PAULI (1922) albumin has a molecular weight somewhere between 1200 and 10,000, casein 1000 to 3000; and globulin 10,000, being bivalent with an equivalent weight of 5000. Then if a normal phosphate buffer system ($KH_2PO_4 \rightleftharpoons K_2HPO_4$, acting as a monovalent buffer) has a buffer index of 0·576 at its maximum, 98 gms. per litre would be the concentration of H_3PO_4; while the concentration of the globulin required for the same maximum buffer index would be 10,000 if it acted as phosphoric acid does or between 10,000 and 5000 if it acts like some complex organic acids, e. g. citric. Taking it at its lowest the ratio is 5000 to 98; and theoretically over 50 times the *percentage* concentration of globulin is required to give a buffer capacity equal to that of phosphate, i. e. one per cent *by weight* of phosphate gives the same buffer capacity as fifty per cent. *by weight* of globulin.

This partially explains how it happens that while 0·98 % (or 0·1 molar) phosphate solution has a maximum buffer index of ·0576, the maximal buffer indexes for 1 % solutions of amino acids and proteins are much lower; e. g. casein and gelatin ·006; albumin ·004; asparagin ·0036; tuberin ·003; gluten ·002. The phosphate as a buffer system, of equal weight-in-volume concentration, is clearly from 9·6 to 29 times stronger, weight for weight, then the amino-acid and proteins mentioned. Taking the equivalent weight of casein as 1000 and phosphate as 98 the ratio is 10·2 : 1 for weights, while the ratio is 1 : 9·6 for the buffer indexes. The difference is easily covered by the experimental errors in the determinations of both equivalent weight of casein and the buffer index of a 1 % casein solution. The agreement is, in fact, remarkably close and indicates that in dilute solutions of proteins adsorptive buffering must be very slight or altogether negligible.

The above considerations make it clear that proteins may be relatively unimportant buffering substances in plant cell sap, or expressed juices, but in the cytoplasm where the proportion of protein may rise to 20 % or more the buffer capacity of this type of material is sure to be of considerable importance.

DETERMINATION OF BUFFERS

Standardisation in the presentation of these complex phenomena is extremely important, not only for comparison of results obtained by different investigators or in different plants or in the same plant at different stages, but also for the extraction from the data of the fullest information. This is the main reason why the inversion of the VAN SLYKE formula by HURD-KARRER is to be deprecated, but even within that author's own presentation we can find a reason in expediency. She states (p. 142) "This ratio $\frac{[\Delta \mathrm{pH}]}{[\Delta \mathrm{B}]}$ is more convenient than the ratio $\frac{\mathrm{dB}}{\mathrm{dpH}}$ suggested by VAN SLYKE, since ΔB is always equal to one, and it is just as useful for the purpose of the present investigation". The making of Δ B equal to the invariable addition of equal volumes of standard alkali of acid ($N/_{20}$ NaOH in this case) is certainly not always possible and involves a departure from the general applicability of the original formula for β as well as from the original mathematical argument. Further the same author in the same paper on the very next page (p. 143) writes "Minimum buffer action occurs at a different point on each curve in fig. 9, but is found, *by reference to the corresponding pH values* (*fig.* 5), to be always near pH 8·0". A buffer index curve presented in the VAN SLYKE manner would have given buffer indices as ordinates and pH as abscissae and there would have been no need to refer back to a previous figure, since the curves would all have shown a dip downwards at a pH ranging from 7·5 to 8·2. The same author (p. 145) misquotes COHN, GROSS and JOHNSON (1919), attributing to them the statement that proteins largely determine the form of the potato tuber-juice titration curve *between* pH 4·5 and 8·5; whereas they really assert (p. 154) that this is the case outside that range, i.e. below pH 4·5 and above 8·5. The actual importance of tuberin buffering in the potato is considered in Chapter XIV of the present monograph, but the above correction is made now in an attempt to prevent the mis-statement getting into the literature.

As HURD-KARRER is one of the very few plant physiologists to take any notice of the VAN SLYKE buffer index formula this criticism has been given somewhat in detail.

Exact work on buffer action in plants has been rather scanty. HEMPEL (1917) contributed our main knowledge of buffering in succulents which have, of course, a special physiology. HOAGLAND and DAVIS (1923) compared titration curves of cell sap and mixed solutions of chloride, sulphate and phosphate. YOUDEN and DENNY (1926) compared titration curves of apple sap and malic acid. LEUTHARDT (cited HURD-KARRER) compared the titration curve of *Mesembryanthemum* and glutamin. Other investigators, including HAAS (1920), GUSTAFSON (1924), CLARK (1917) etc., have recorded buffer action in the form of titration curves of plant juices but have given no records of the actual buffering substances (see also NEWTON 1923 for titration curves only).

MARTIN (1926, 1927), INGOLD (1929) and ARMSTRONG (see Chapter XIX) have recorded buffer values and have, like HEMPEL, identified quantitatively as far as possible the buffer systems present in sunflower, bean, potato and fungi, thus extending HEMPEL's methods to more or less ordinary plants.

The best procedure, in the opinion of the writer and his colleagues, is to plot carefully and record a titration curve upon a large scale and to construct a buffer index curve from that, using the VAN SLYKE formula according to the details given on p. 69 above. The dB can then be taken for pH as unity or as 0·5 or 0·2 according to the degree of accuracy desired. It is better for actual identification of buffers to take dB per 0·5 change of pH, because the characteristic maximal points of buffer action are obscured if these points are less than 0·5 pH apart and too great a degree of accuracy in the β curve may give a wrong impression in the search for the buffering substances. By noting the position of the maxima of the β curve with relation to the pH scale, it is comparatively easy to fix upon possible buffering substances in a mixed plant sap. Further, since the *maximum* buffer index varies directly as the concentration of the buffer system, the β curve of a plant juice may indicate not only the nature of the buffer but also the concentration of that buffer (see Chaps. XII—XIV and XIX). The buffer action of mixed buffers being, completely and clearly additive[1]), it is possible by actual quantitative analysis to resolve the buffer index of a sap in any particular range into those of the constituent buffer systems acting in that range (see *Collybia velutipes* in Chap. XIX).

The possible buffer-systems having been *indicated* by the β curve, quantitative analysis should be used where possible in order to confirm or confute the suggestions of the curve. For example, if the maximum β be found between pH 6 and pH 7, phosphates and the bicarbonate-carbonic acid system are possible buffer systems, but if the curve does not show a dip between pH 5 and 6 the probability is either a plain phosphate system or the presence of an additional system with a maximum at a lower point on the pH scale, the bicarbonate-carbonic acid system being rendered less probable. Such a point can be settled only by quantitative estimation of the phosphate present. If the quantity found be sufficient to account for all the buffer index between pH 6 and pH 7 the other system is eliminated. The same procedure applies to organic acids which occur more or less in groups, each group having the maximal buffer indices within a definite pH zone e. g. pH 4—5, and pH 3—4. Quantitative analysis should be used in order to identify the acid and to check the indications of the β curve (see Chapter XIX).

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[1]) INGOLD unpublished work, and Chap. XIV.

At the same time, if investigators do no more than record the buffer index curve they make a *clear* contribution to our knowledge, and they make subsequent comparisons and investigations so much the easier.

PART III

RESULTS

CHAPTER X

GENERAL SURVEY OF TISSUE REACTIONS

In considering the data available it may be convenient to classify them under the following heads. — 1. Natural Indicators; 2. Juices of parts; 3. Meristems; 4. Reactions associated with Tropisms; 5. Stomata; 6. Tissues in general.

1. NATURAL INDICATORS

Extracts of various plants and animals have been used as indicators *in vitro*, e.g. litmus, orchil, cochineal, turmeric, alkannin, logwood, red wine, mimosa flower extract, etc. (see CLARK 1928 p. 86), but we are here concerned rather with these and others as indicators *in vivo*.

Petals. — SCHWARZ (1892) and WILLSTÄTTER (1914) observed that the anthocyans of flowers acted as natural indicators (see p. 39) WILLSTÄTTER's data are pH 5·5 for rose petals and pH 7·2 for cornflower corolla. HAAS (1916) used anthocyans as natural indicators and records the following pH values — *Viola tricolor* 4, *V. odorata* 5, *Primula chinensis* 6—7, *P. obconica* 6, *Hyacinthus* blue 4·5, ditto red 7, *Scilla* 4·5, *Cichorium intybus* 3, *Pelargonium* 8, *Browallia speciosa* 6.

JACOBS (1922) used Rhododendron blossom in an investigation and records a natural indicator which is red, as in the flower, below pH 7·0 and changes to violet or blue at pH 8·0 and above. ATKINS (1922) records plants of *Hydrangea* as pink or blue on soils of various reactions, usually pink when the soil pH was above 7·5 and blue when it was 6·0 or 5·75 but with both colours at 5·9, 6·2 and 7·3. Atkins, however, found that the anthocyan in this case was *not* an indicator and that the expressed sap of the petals of both colours (by a drop comparison method) was of pH 4·0—4·2. He concluded from further work that the blue colour is due to iron but "It is possible, however, that the aluminium, as well as the iron, may form a blue complex with the anthocyanin, which is pink in the absence of excess of these salts".

Smith (1923) used petals of *Ipomea Learii* and records the anthocyan as red in the bud (pH 6), blue when opening (pH 7·8) and red again when fading (pH 6). Water saturated with carbon dioxide was found to change the virage from blue to red in a reversible fashion, although "strong" acids (pH 5) were without influence on the tint. Mevius (1924) and others have also used natural indicators for investigations on effects of external conditions.

Indicator anthocyans doubtless occur in many other petals. It is amusing, for example, to exhibit the field scabious, *Scabiosa arvensis*, to one's friends as a pinkish lilac blossom and then to turn it bright green by fumigating it with alkaline tobacco smoke! The observations made by Atkins, however, should make it clear that red-blue anthocyans are not always indicators.

Nectaries — McClendon (1914) observed a natural indicator in the nectar glands of *Vicia faba*, which showed red-acid, blue-alkaline, and gave rise to colour changes during the functional activities of the glands. The actual reaction is not given.

Other Plant Parts — Walbum (1913) used an extract of red cabbage. McClendon (1914) also notes that the red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) contains a natural indicator. Haas (1916) records red cabbage leaves as pH 6—7, radish root as pH 2 – 3, and red beet-root pH 3. Crozier (1916) found that the red berries of *Lantana involucrata* have an indicator with a green to yellow virage above pH 6·6, that the berries of *Opuntia* show a natural virage indicating pH 9, while the fruits of *Randia aculeata* show green above pH 8·5 and red below that reaction. Schley (1913) used a natural indicator in *Vicia faba*, and the writer has observed the acid-green, alkaline-brown indicator of the bean seed, also the acid-colorless, alkaline-yellow indicator of the maize.

2. JUICES OF PLANT ORGANS

The beginnings of our knowledge of this subject are lost in antiquity, beyond the origin of the proverb "The fathers have eaten sour grapes, and the children's teeth are set on edge", which proverb was old in Ezekiel's time (18·2). **Heyne** (1815) records that the leaves of *Bryophyllum calycinum* are "acid as sorrel in the morning but lost their sour taste as the day wore on". **Link** (1820) confirmed the phenomenon, using litmus paper. **Kraus** (1883) found similar effects. The matter of *sour* taste has been correlated with hydrion concentration by **Richards** (1898), **Kastle** (1898), **Kahlenberg** (1900), **Paul** (1915— 1916), **Harvey** (1920) and **Hampshire** (1921). **Gustafson** has recently (1924—1925) re-examined the diurnal changes of *Bryophyllum*, using the expressed centrifuged juice of the young parts and finds (1925) pH 3·75 in the morning passing to pH 5·3 in the evening. **Hempel** (1917), **Ulehla** (1927—1928), and others have investigated succulents in some detail (see Chapters XI and XV).

The observations of the reaction of normal plants begin later than that of those acid succulents[1]). **Gaudichaud** (1848) noted that, using 'les papiers chimiques' all the fluids, raw sap and food solutions, of the plant were usually acid. **Sachs** (1862) also found that the sap of most plant cells is acid or *sour*. **Wagner** (1916) drew the juice from the chlorenchyma of leaves by means of a fine glass pipette and used a microcolorimetric method with lakmosol as an indicator. He records for the leaf juice of *Sinapis alba* pH 5·5, *Brassica oleifera* pH 5·65, *Sempervivum Hausmannii* pH 5·4, *Solanum tuberosum* pH 5·8, with pH 5·9 for the tuber juice of the potato. **Wagner** also infected the first two with *Pseudomonas campestris*, the *Sempervivum* with *Bacillus vulgatus* and the potato with *Bacillus phytophthorus*, and observed an initial rise of about 0·1 in pH followed by a decrease in pH of 0·2 to 0·6 units. If the plants recovered, the pH returned to normal, while if it succumbed there was another rise in pH during several days, ending in an abrupt fall when the tissue died. **Harvey** (1920) found a similar rise in *Ricinus communus* and a larger rise in *Beta vulgaris* infected with *Bacillus tumefaciens* (see p. 88).

Hurd (1924) found in wheat that "infection by mildew, when severe enough to visibly affect the vigour of the plant, results in an abnormally high acidity", and concludes from her work that "varietal resistance to stem rust is not related at any stage of development to titratable-acid or hydrogen-ion concentration. High acidity of the juice does not hinder attacks of the stem rust organism, ... low acidity does not pre-dispose to the disease", either in resistant or susceptible varieties. The same worker (HURD-KARRER 1925) found no correlation again between pH or total acidity and resistance of wheat to *Tilletia Tritici.* WALKER (1923) had found the same for several varieties of onions and *Colletotrichum circinans*; and ARRHENIUS (1924) agreed for yellow rust wheat. SMITH (1924), on the other hand, reports that plants with very acid juices are immune to *Bacterium tumefaciens*. All this is in quite reasonable agreement with what is known of the effects on fungi and bacteria respectively of the pH of the medium external to the parasite.

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[1]) For further data see Appendix III.

REISS (1925) records H-electrode data, for the potato, of expressed juice and also an extract of the purée with two volumes of water. The normal tuber gave pH 6·01—6·21, while the tumour of a tuber infected with *Synchytrium endobioticum* gave pH 5·59—5·85.

HEMPEL (1917) also investigated the juice of lupine tops, finding with lakmoid pH 5·9 and electrometrically pH 5·78—6·03, with the seedling juice constant at pH 5·9. She also found lemon juice to be pH 2·19—2·25 and the pitcher fluids of *Nepenthes* to vary (electrometric data); *N. paradisa* being constant at pH 3·20, *N. Tiveyi* pH 4·61—5·87, and another species pH 5·51, the latter two rising with hydrogen transfusion to pH 8·15 and 7·97 respectively. KAPPEN (1918) found that root saps were only very slightly acid and gives a series in order of increasing acidity thus-wheat, barley, oats, rye, dwarf bean, horse bean, lupin, mustard, buckwheat. STOKLASA (1924) using both electrometric and indicator methods found for the pressed juice of roots the following pH values —

Triticum vulgare and *Hordeum distichon* 6·9;
Secale cereale 6·8, *Avena sativa* 6·6, *Zea mais* 4·4;
Medicago sativa 6·8, *Trifolium pratense* 6·6;
Beta vulgaris 6·4, *Fagopyrum esculentum* 6·2;
Solanum tuberosum 7·0.

All these values, except maize, are very high, probably on account of the hydrogen-electrode errors.

Hoagland (1919) used the hydrogen electrode method; the plant material (barley) was comminuted, frozen and pressed. The juice from the tops gave a definite and constant value of pH 5·68—5·85; that from the roots was pH 6·12—7·08 (cp. Stoklasa 6·9) but a decrease in acidity was noted during the measurement of the root juice (cp. *Nepenthes* above) which was suggested to be due to reduction of NO_3 and the absence of a sufficient buffer effect.

Truog (1920) found that liming of the soil reduced the actual acidity of plant juices. This was further investigated by **Haas** (1920), using a small hydrogen electrode. He found that equilibrium was more rapidly attained with small quantities than with larger quantities of fluid. He also found that sometimes a small degree of alkalisation occurred on allowing the fluids to stand for half an hour or more, for fresh medium red clover pH 6·02 became pH 6·06; and also that on standing for about six hours there was an acidification, pH 6·12 to pH 5·94 or pH 6·12 to pH 5·82. Using the same juice he found a variation in the second decimal place which amounted to 0·02—0·04. **McClendon** and **Sharp** (1919) had previously recorded a change in the pH of carrot juice from 5·85 to 5·73 after standing twenty minutes. These changes may be due to (a) chemical change or (b) the carbon dioxide of the air combined with a low buffer capacity in that pH range. The main investigation by **Haas** was on the effect of liming, and the results are given in Table III as a summary of the reaction of juices. A slight increase in acidity with age was found to occur in corn seedlings. **Haas** (1920) also gives further data for buckwheat tops, partially flowering and in seed, as pH 4·82, with "a marked buffer action". He quotes as an extreme example of the gradient in reaction, sweet clover which shows pH 8·00 for the upper three inches of shoot, pH 7·04 for remainder of leaves, 6·68 for remainder of stem to 2 inches above soil, 6·46 for lower two inches of stem, and upper two inches of root, 5·82 for six inches of root below the upper portion. No other species was found to show this gradient.

The juice of sweet clover tops was found to be alkaline by this electrometric method and was also found to contain about 50 % more carbon dioxide than the relatively acid tops of medium red clover. This is an interesting example of the study of the hydrogen transfusion of the electrometric method as affecting the

Table III

From HAAS (1920) — Table 4 (p. 355).

Plant	Actual Acidity	Actual Acidity of Juice		Total Acidity
		Limed pH	Unlimed pH	
Alfalfa tops	Decrease	6·19	5·99	Decrease
Alfalfa roots (Baltic no.550)	Increase	6·12	6·21	
Alfalfa roots (Common South Dakota no. 363) .	Decrease	6·12	6·06	
Alsike clover roots	Decrease	5·84	5·58	
Alsike clover tops	Decrease	6·19	5·28	Decrease
Barley tops	Decrease	5·72	5·62	No difference
Buckwheat (entire seedlings)	Decrease	5·97	5·48	
Corn tops	No difference	5·19	5·19	Slight increase
Corn tops	No difference	5·48	5·48	
Field peas tops	Increase	6·53	6·80	
Garden bean tops	Increase	5·65	5·97	Increase
Lupine, yellow tops . . .	Decrease	5·63	5·31	
Lupine, yellow roots . . .			5·80	
Medium red clover roots .	Increase	5·87 5·82	5·91 5·88	
Medium red clover roots .	Decrease	6·12	5·94	Decrease
Medium red clover leaves.	Decrease	6·19	6·02	No difference
Medium red clover stems and petioles	Decrease	5·95	5·63	Decrease
Medium red clover tops .	Decrease	5·92	5·81	Decrease then increase
Mustard, white, roots. . .	Decrease	5·91	5·62	
Mustard, white entire plants	Decrease	5·78	5·48	
Oat plants	Decrease	5·67	5·65	Slight increase
Serradella tops.	Increase	5·74	5·94	Decrease then increase
Timothy tops	Decrease	6·17 6·19	6·14 6·12	
Winter wheat crops . . .	Decrease	6·33	5·95	
Winter wheat, leaves and stems, no heads	Decrease	6·12	5·77	

reaction of plant juices. We have already seen that small quantities were used in order to obtain equilibrium rapidly. All these records by **Haas** would appear to be, like those of most other electrometric determinations, data concerning the residual reaction of the juice after the removal of carbon dioxide. No evidence is presented which proves that the natural sap of sweet clover tops is less acid than that of red clover tops; we are, in fact, given a certain amount of evidence, in the table of carbon dioxide values (p. 362) that the hydrogen-electrode method shows a greater error with sweet than with red clover tops, because there is more carbon dioxide to be removed.

The Effect of liming on Plant Juice Reactions. -- This problem has been studied by others. **Bryan** (1919) found that in general the sap pH followed the soil pH but in corn tops there was no change, and that in general the pH of the root sap was more closely correlated with that of the soil than was the pH of shoot sap. **Truog** and **Meacham** (1919) found lupine to be an exception to the same general rule of higher pH with liming. **Clevenger** (1919) found root sap pH correlated with soil pH, but that shoot sap pH was lower on limed than on unlimed soils. **Haas** (1920) gives the results in Table III and others; the general rule holding with exceptions as before.

Bauer and **Haas** (1923) using various fertilisers, found variations from pH 5·31 to pH 5·95 in corn stalk sap and 5·31 5·49 in corn leaf sap. **Hurd** (1923) found a correlation between relative vigour and pH of corn stalk sap -- Series I April May, very good, pH 5·5; Series IV June—July, very good, pH 5·5; Series II May—June poor, pH 5·3; Series III May—July, very poor, pH 5·1. There was not the same correlation between leaf sap pH and vigour; the average pH values for leaf saps being 5·42 for vigorous and 5·43 for poor growths of the various strains; whereas the corresponding averages for stalks were pH 5·51 and pH 5·19.

Arrhenius (1922) and many others have studied the effect of soil pH on growth without giving data for internal pH and these results belong to the other section of the subject. **Newton** (1923) using barley, peas and beans found that the internal juice pH was not decreased by limiting the supply of calcium and this was confirmed for the juice of tomato by **Dustman** 1925.

These results indicate that the relations between soil pH and internal (juice) pH are complex. Probably they cannot be elucidated without a considerable amount of work on actual tissue reactions and accurate determinations of buffer effects along the more modern lines.

ETIOLATION ETC.

Hempel (1911) found that the absence of chlorophyll from lupine seedlings had no effect on the pH of the juice, but Haas (1920) found for corn seedlings — green tops pH 5·52, green-tinted white tops pH 5·85, white tops pH 6·16. This difference may or may not be due to an increasing carbon dioxide error with the hydrogen electrode, there is no evidence in this paper, but see below. Further work by Bauer and Haas (1922) led to the conclusion that the growing plant seems to be able to adjust or regulate internal changes readily.

Further work on the acidity gradient in plants was done by Gustafson (1924) who found a pH gradient in many plants, which "is not due to unequal dilution of the cell contents nor to unequal amount of CO_2 dissolved in the juice." Data for the pressed juice of sunflower as determined by the hydrogen electrode are upper leaves 6·7, lower leaves 6·4; upper stem 6·0, lower stem 6·0. Similar figures are given for other plants. Since Gustafson attempted to avoid the error of varying carbon-dioxide content, this evidence of a pH gradient is somewhat more reliable, but the actual reactions as given are still subject to the errors of pressed juice and are only comparative data. Similar data are given by Mukerji (1927) for expressed leaf juice of *Mercurialis perennis* — lower leaves pH 5·8–6·0 and uppermost pH 4·8—5·0. In a further contribution (Gustafson (1924 b) finds no constant relation between total and actual acidity in *Zea mais*, *Cucurbita maxima*, *Helianthus* sp. and *Bryophyllum calycinum*. He confirms very definitely the observation that the total acidity does not control the actual acidity of plant sap, finding a greater *total* acidity in young parts concomitant with a lesser *actual* acidity than in older parts of the same plants. The gradient of total acidity in the reverse direction had previously been demonstrated by Kraus (1880).

In the years 1921—1923 Atkins published a series of papers which were important contributions to various aspects of hydrion concentration in relation to plants. He recognised the presence of errors in both electrometric and colorimetric methods, due to the manipulation and mixing of saps in order to obtain free juice. Using a drop method with expressed juice he obtained the following pH values —

Triticum seedlings — roots 6·8, white leaf bases and young green leaves 5·4,
Avena sativa — roots 6·8, leaves 5·4,
Oryza sativa — roots 6·8, leaves 4·8, first and second internodes of stem 5·0
Ricinus communis — stem 4·6, leaves and young flower 4·8, a section method on young capsule showed-walls 4·8, immature seeds 5·4, also pollen sacs 5·4.

HARVEY (1920) had previously recorded H-electrode data for *Ricinus communis* thus — tumour 5·77, stem 5·52, leaves 5·6, and also *Beta vulgaris* — normal root 5·8, tumour produced by *Bacillus tumefaciens* 6·35.

RONCATI and QUAGLIARIELLO (1921), GRAY and RYAN (1921), BREWSTER and RAINES (1922), and others have given a few scattered data concerning the pH of plant juices on similar lines. PEARSALL and EWING (1924) record data for expressed juice of reserve organs; potato pH 5·4—5·6, carrot pH 6.

ZACHAROWA (1925) gives various pH data for parts of roots (cited by MEVIUS) the actual figures vary from pH 5·0 to pH 8·0 but considering the technique (see p. 37), they need not be quoted.[1] DUSTMAN (1925) gives pH values varying from 5·50 to 5·61 for the pressed juice of the tomato. DOYLE and CLINCH (1926, 1928), using ATKINS' drop method, obtained some interesting pH values for the expressed leaf-juice of conifers *Abies pectinata* 3·7, *Cedrus* spp. 3·5—3·6, *Picea* 3·7, *Pinus* spp. 3·6—3·8, *Pseudotsuga* 3·7, *Tsuga* spp. 3·4; *Cupressus* spp. 5·1, *Thuja* spp. 5·1—5.2, *Juniperus* spp. 5·0—5·4; *Sequoia* 3·6, *Cryptomeria* 3·8, *Sciadopitys* 5·0, *Araucaria* 3·9; *Podocarpus* 5·1, *Cephalotaxus* spp. 5·3—5·4, *Torreya* 5·6, *Taxus* spp. 5·4. With exception of *Sciadopitys*, all the Abietineae and Taxodineae are in the range of pH 3·4—3·8, the Cupressineae show a range of pH 5·0—5·4, and the Taxaceae pH 5·1—5.6.

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1) See also PFEIFFER (1929) where the R.I.M. ranges are corroborated.

Ulehla (1927, 1928) records for pressed sap of *Opuntia phaecantha* pH 1·4 to pH 6·0 according to the time of day; *Rheum undulatum* leaf pH 2·95—3·05; leaf parenchyma of *Nymphaea alba* pH 3·1.

Nemec (1925) used a special technique, obtaining results which appear to have a comparative value but which may have very little relation to the reaction in the tissues concerned. He used seeds, crushed, added to distilled water (5 gms.—100 cc.) with 2 gms. toluene as preservative. After extracting thus for 48 hours at 27°, he filtered and determined the pH by means of a H-electrode. He concluded that acid-soil plants show a low pH and *vice versa*; thus.

	Soil optimum	pH Seed Extract
Agrostis canina	5·2	4·9
Poa pratensis	6·2	5·6
Festuca pratensis	6·9	6·1
Lupinus luteus	calcifuge	4·5
Hordeum distichum	calcicole	6·4

3. MERISTEMS

The earliest observations of the reaction of meristems appears to be the observation of Sachs (1862) who found that most cell saps were acid but that certain tissues always exhibited an alkaline reaction — "nämlich in den dünnwandigen Zellen, welche bei vollständig ausgebildeten Gefäßbündeln krautiger Pflanzenteile zwischen dem Baste und den Gefaßröhren liegen." Later authors (Pearsall and Priestley 1923, Priestley 1928, see Weber 1924) have supposed a certain pH value for meristems as lying, like the tissue, between acid values on one side and less acid values on the other. No names are given of the plants discussed and these generalisations do not appear to rest securely upon any wide range of observations. Some of the data given in Table VI would form support but there are too many exceptions, especially in the 'all acid' families. These authors quote Atkins for acidity in the xylem and Sachs for alkalinity in the phloem. Readers are referred to Table VI, where records of the variation which actually occurs can be seen. There is no experimental evidence that the pH of one tissue can really affect the pH of an adjacent tissue, but there is a large body of evidence in support of the independence of tissue or cell reactions. The occurrence of very acid cells and less acid cells in a mixed cortex with only the walls between, and of sharply defined acid tissues adjacent to less acid tissues, e.g. epidermis, pericycle, etc., shows clearly that the internal pH of any particular cell depends upon the metabolism of that cell and not upon the pH of adjacent cells. Membrane buffer effects and experiments with varying reactions of the external medium in relation to the internal pH of cells supply a satisfactory explanation of this independence of cell-reaction. This meristem hypothesis also involves an isoelectric point for living plant proteins, another very hypothetical property of the cell which is discussed in Chapter XVI. In addition to all these points we have **Herklots'** interesting observations (1924) on the healing of cut potatoes with varying pH values of the medium, during which he found that alkalinity of the buffered medium (especially from pH 7·5) promotes suberisation but retards meristematic activity and that after a suberised block has been formed acidity (pH 6·5 up to pH 4·6, the limit of the experiments) promotes phellogen activity, but retards its subsequent suberisation (see also **Pfeiffer** 1925b, Section II). **Samuel** (1927), using a micro-hydroquinone electrode, obtained, results which lend no support to the application of this theory to the 'shot-hole' meristems of diseased leaves[1]).

4. REACTIONS ASSOCIATED WITH TROPISMS

Greenwood and **Pearsall** (1926) state that "It has been found by **Schley** (8, 9) that the upper and lower sides of the stem of broad bean (*Vicia faba*) when placed horizontally show differences in reaction (pH value[2])) and although **Phillips** . . . was unable to find significant differences for *Vicia faba*, yet he obtained similar results for corn."

Schley (1913) in the paper referred to above states (pp. 485—86) that "Since this method measures the titration value but not the H-ion content of the acid, the results are not directly comparable with the work of **Fischer**, whose conception, besides the acid change, involves also changes in the amount of salts and the nature of the colloids, which have not been undertaken in this work. So far as the results indicate, however, they show no difference which would explain curvature on the basis of increased acidity, since at the time visible curvature begins the two flanks are of equal acidity."

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[1]) See also **Pfeiffer** (1929) who finds the cambium similar in reaction to the phloem, or even less acid.

[2]) G. and P.'s brackets.

Schley (1913) titrated with N/10 NaOH using the natural indicator of the bean as indicating the end-point; a test plate method of determining the end-point with phenol-phthalein in preliminary experiments being mentioned. Schley (1920) gives no new acidity data but refers to the earlier (1913) results. The facts, therefore, are that Schley (1913) found changes in total acidity of the two sides of a geotropically stimulated stem but did not then consider these differences as a possible cause in geotropic curvature, and has not recorded any pH data in this connection in either of the two papers mentioned.

Phillips (1920) found that the *titration* acidity of geotropically stimulated corn shoots was greater on the convex side of the curve, and that both titration acidity and pH in *Vicia faba* were sometimes more and sometimes less on the convex side of a geotropically stimulated shoot, from + 0·080 to —0·143 as determined by a H-electrode method on pressed juice.

The only available consistent data concerning the hydrion concentration of the two flanks of geotropically stimulated organs are, therefore, those of Greenwood and Pearsall (1926). Using an indicator method on the juice of 3 halves 5 cms. long, crushed in 3 ccs. distilled water, these authors found a consistent difference of about 0·2 in the pH of this fluid when the stems and roots were stimulated by gravity. Their results may be summarised thus —

	Stems		*Roots*	
	Upper	Lower	Upper	Lower
Vicia faba	6·2—6·3	6·0 6·1	6·15 6·3	6·4 6·5
Pisum sativum	6·4	6·2	6.2	6·4
Helianthus annuus (hypocotyl)	5·9	6·2	6·2	6·4

These same authors record pH values for the exuded sap from these shoots thus — *Vicia faba* 4·8; *Pisum sativum* 4·7; *Helianthus* hypocotyl 5·7. They also give data for the effect of root exudates upon the pH of distilled water, claiming for these "presumably a slightly alkaline reaction".

Since all the pH values given are within the effective range of carbon dioxide and the method used, crushing in distilled water, involves destruction of the cells and a mixing of the tissue fluids we are still without any valid data concerning the effect of gravity upon the reaction of the tissues in stimulated organs.

5. REACTIONS ASSOCIATED WITH STOMATA

The history of stomatal physiology has been very adequately reviewed by WEBER (1923b, 1926) and later by SCARTH (1927). WEBER's suggestion of the action of carbon dioxide in controlling the pH of the guard cells and SCARTH's experimental demonstration of the phenomenon, 'clinched' by the use of WEBER's Z. I. M. in the determination of the internal pH of the guard cells under varying conditions; these have resulted in our present rather satisfactory knowledge of the stomatal apparatus as a self-regulatory mechanism, (see also WEBER 1923a, SAYRE 1926, SCARTH 1926 and GICKLHORN 1928).

According to SCARTH — "There seems to be little room for doubt that the characteristic changes in the guard cells are due to fluctuations of H-ion concentration within them and that these fluctuations are such as might be produced by the normal changes in concentration of CO_2." The range varies with the species and extends from pH 4·5 to pH 7 approximately, all within the range of carbon dioxide action. The reversible hydrolysis and synthesis of starches which have been suggested as partial causes of the phenomena are "vastly too slow to account for the rapid response of movement". Changes in intermediate saccharides may account partly for the rapidity, but SCARTH favours the more rapid changes which occur in the hydration capacity of an amphoteric colloid in the sap of the cell. He gives strong evidence for the presence of such a colloid actually in the sap of guard cells. This last factor accounts not only for a large part of the rapidity of the guard cell movements, but also for the fact that the stomata open both under relatively acid and relatively alkaline conditions. Normally the chlorophyll-containing guard cells close the stomata under assimilating conditions (relatively alkaline) and open them under shade conditions, but at night or in prolonged darkness hyperacidity may result in the opening of the stomata. According to our present knowledge, therefore, the carbon dioxide content of the guard cells controls the pH of these cells and the pH controls opening and closing of the stomata. According to Scarth, the principal function of the stomata is to regulate that very factor which is presumed to regulate them, viz. the concentration of CO_2 in the leaf, especially in the guard cells.

6. TISSUES IN GENERAL

Determinations of the internal reaction of plant cells occur scattered in the literature as incidental information, but it is doubtful if such data are critical. ANGERER (1920) and BALINT (1924) attempted to determine the internal pH of bacterial cells, but the methods used were not reliable.

Algae.

ROHDE (1917) found *Spirogyra* sap of pH 5·5—5·9 during the day and pH 6·73 during the night, using both indicator and H-electrode methods. CROZIER (1919) found *Valonia* sap to be usually of pH 6·0—5.9, varying from pH 5·0—6·7. LAPICQUE (1922—23) found the pH of *Spirogyra* below 5. PEARSALL and EWING (1925) record pH 6·2—7.0 as the normal sap reaction of *Spirogyra*. The later authors do not record the time of day of their determinations and ROHDE'S results are the only critical data.

ATKINS (1922) using his section method records pH 7·3 for *Laminaria* stipe; pH 7·2 for the medullary tissue of the receptacle of *Fucus*; pH 6·6 for the stipe and pH 6·9 for the disc of *Himanthalia*; pH 7·0 for *Ulva*; pH 7·0 for the diatom *Skeletonema*.

HOAGLAND and DAVIS (1923) record pH 5·2 as sap reaction of *Nitella*, using both colorimetric and electrometric methods. TAYLOR and WHITAKER (1927) using a micro-H-electrode found an average value of pH 5·47, but consider pH 6·16 to be nearer the true value for the sap of *Nitella*. There is possibly a diurnal variation here, as in *Spirogyra*, and both figures may be correct.

MISS M. CLAPHAM in this Department has examined *Laminaria* tissues using the R.I.M. and reports as follows. —

While finding it difficult to get full indications, MISS CLAPHAM gives the following values for the contents and walls of the ventral region of the thallus. — *Laurencia caespitosa* pH 4·0 *ca.*; *Laurencia pannatifida* pH 4·0 *ca.*; *Chondrus crispus* within the range pH 5·9—4·4; *Dictyota dichotoma* within the range pH 6·2—4·4.

Laminaria digitata	outer cortex	inner cortex	medulla
stipe, young	5·2 4·8	6·8 6·4	6·2 5·9
	5·9	6·8 6·4	
	5·9	6·2 ca.	5·9
stipe, medium age	5·2 4·8	6·8 6·4[1]	5·2 4·8
stipe, old	5·2	6·8 6·2	5·9 5·6
hapteron, young	5·9		6·8 6·4
hapteron, old	5·9		6·8 6·2
Laminaria saccharina			
stipe, very young	5·2 4·8	5·9	5·6
stipe, young	5·9	6·8 6·4	5·9

Fungi.

Although there are numerous observations of the interaction of fungi and fungal media, there are few records of internal reaction in this group. HERRMANN (1879) records a blueing of litmus paper by the plasmodium of *Aethalium*, and there are at present no other published records known to the writer. The following data (Table IV) have been obtained by J. I. ARMSTRONG in this Department, using the R.I.M., with BTB and phenol red PR as additional indicators.

C. T. INGOLD, also in this Department, determined the pH of the sap exuded on puncturing the sporangiophore of *Pilobolus crystallinus*, by means of drop comparisons, applying the R.I.M. method of interpretation. The sap was alkaline to B.P.B., B.C.G., B.A.N., M.R., D.E.R., B.C.P., B.T.B. and to neutral red; pink ($pH > 7·0$) with phenol red (P.R.) and yellow (pH 7·4) with cresol red. The actual pH lies, therefore, in the range pH 7·0 7·1.

It will be clear from an inspection of Table IV that the tissue reactions in the fungi examined are higher than is common in flowering plants (see Table VI) and that there is little differentiation. The stipe of *Hypholoma fasciculare*, of *Clitocybe*, of *Lactarius* and of *Panus*; the hymenial layers of the immature *Armillaria mellea* and of *Cortinarius violaceus* are the chief variations found within the same fructification. *Leotia* showed mixed tissue-reactions.

Bryophyta.— There are no previous records of internal pH values for this group, but a few of these plants have been investi-

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[1] The inner cortex showed a band of pH 5·2 4·8.

Table IV

Species	Soil pH	pH Values for Tissues			Notes
		Stipe	Pileus	Hymenial layers	
Coprinus atramentarius	6·5	D	D	D	
„ micaceus (young)	7·6—7·8	b	b	b	
„ „ (mature)					
Armillaria mellea . . .	5·5—6·0	C	C	C	
„ „ (young)	5·2 ca.	a	a	c	
„ „ (mature)		a	a	a	
Clavaria rugosa	6·0—6·5	C	—	C	T. S. fructification
„ corniculatus .	7·0—7·2	B	—	B	„ „ „
Typhula incarnata . .	6·0—6·2	a	—	—	„ „ „
Mycena vulgare . . .	—	a	a	a	fleshy forms
„ pura	6·8—7·0	a	a	a	
Lactarius blennius . .	5·5—6·0	b	c	c	
Cortinarius violaceus .	5·5—6·0	C	C	a	
Amanita muscaria . . .	—	C	C	C	
Hypholoma fasciculare	6·0—6·2	e	a	a	
Agaricus campestris . .	5·6—5·8	a	a	a	
Clitocybe laccata . . .	6·0—6·5	C	a	a	leathery forms
Panus torulosis	5·5—6·0	C	b	b	
Collybia radicata . . .	5·5—6·0	—	a	a	
Polystictus versicolor .	—	a	a	a	tubes. woody forms
Polyporus sp..	—	a	a	a	
Xylaria hypoxylon . .	—	a	—	-	
Leotia chlorocephala .	5·5—6·0	C	Ce	e	centre of pileus 6·2, periphery 5·6
Helvella crispa	6·5—7·0	C	C		

The letter B is used for pH 6·2—5.9; C for pH 6·2 approx.: D for pH 6·8—6.2, in extension of the R.I.M. notation with the use of bromothymol blue, BTB., and phenol red PR. a - ca. pH 5·9, b pH 5·9—5·6, c ca. pH 5·6, e - - pH 5·2—4·8.

gated in this Department by Miss M. J. LYNN. Differentiation is not easy to find but the following have been noted.

Marchantia polymorpha. — Thallus except air-chambers pH 5·2—4.8; rhizoids, both types pH 4·0.

Fegatella conica. — thallus except air-chambers pH 4·4; rhizoids, both types pH 4·0.

Polytrichum commune. — Leafy stem shows - epidermal walls k, contents pH 4·4—4·0; cortex pH 4·4 - 4·0; leptome contents pH 6·2—5·9 (B); hydrome mantle contents varied from h to f (pH 5·2—4·0); hydrome walls k.

Pteridophyta. — There are again no previous records of internal pH values for this group, and a few of these plants have been similarly investigated in this Department by Miss M. J. LYNN. Differentiation was sometimes more extensive, and in *Equisetum*, as in *Polytrichum*, was very interesting.

Equisetum maximum. — Taking S for self-colour the following values were indicated —

	Epid.	O. C.	I. C.	cells lining vall. canal	En. & Pe.	Phl.	Proto-phl.	lat. xyl. walls	Pith
Stem Internode .	S	S	<5·9	e	S	b	C'	l	e
Rhizome	S	S	d	e	e	a	C'	l	e
Strobilus young axis	S	e	e	e	e	a	C'	e	e

The sporangiophore, very young, — showed rows of cells in stalk and some cells in head with very acid (pH 4·0) contents.

Selaginella martensii. — The stem showed the epidermis and all the cortex in the range pH 5·2 - 4·8; pericycle and phloem pH 5·9; xylem walls i.

Aspidium filix-mas. — Young rachis was all in the range pH 5·2—4·8, with no clear differentiation. The rhizome was exactly the same.

Asplenium viviparum. — The tip of the young rachis showed epidermis and ramenta pH < 5·2, cortex at pH 5·6; endodermis, pericycle and phloem pH > 5·9; protoxylem walls h. The base of the young rachis was similar except that the endodermis, pericycle and phloem showed pH 5·6. The base of the old rachis was more acid; with the epidermis self-coloured, outer and inner cortical parenchyma contents in range e, sclerenchyma walls k; endodermis, pericycle and phloem pH < 5·2 (Z).

Gymnospermae. — The only published data of internal pH for this group appear to be those by DOYLE and CLINCH on expressed juice of leaves, see p. 88.

Miss LYNN has investigated a few examples of this group and reports as follows.

Pinus austriaca — stem (two years old) — walls of epidermis, hypodermal sclerenchyma and xylem **k**; phloem parenchyma and pith pH 5·2—4·8; some cells of inner cortex pH 4·4 *ca.*; leaf—walls of epidermis, hypodermal and bundle sclerenchyma and xylem **k**; contents of endodermis pH $<$ 3·4; mesophyll self-coloured except some cells clearly at pH 5·2—4·8.

Taxus baccata form *fastigiata* — stem (two years old) — walls of epidermis and xylem **k**; contents of epidermis and phloem parenchyma pH 5·2—4·4; contents of many cortical cells pH 4·4; contents of many cells of pith pH $<$ 3·4 : leaf-cutin and xylem walls **k**; acid cells pH (4·0) scattered in epidermis, mesophyll and bundle sheath.

Flowering Plants. — Tissue reactions in the flowering plants had been recorded previous to 1926 mainly by ROHDE, ATKINS and PFEIFFER. A general survey was made in this Department during the years 1922 to 1927, and some of the results have been published as a series in Protoplasma (see SMALL, REA, MARTIN, INGOLD). These results form the basis of this and the next few chapters.

MICHAELIS and KRAMSZTYK in 1914 gave data for certain animal tissues. ROHDE (1917) followed with one of the most careful accounts yet published of tissue reactions. He used very dilute indicators, ·5% indicator solutions diluted 500 times with water, on immersed sections, and also a H-electrode method on filtered pressed juice. The indicators used were neutral red, methyl red and methyl orange, and a plasmolysis test was applied for the final living condition of the cell. His results may be summarised as follows —

1. Plant cells with a neutral reaction —
   pressed juice and H-electrode — carrot 7·35, asparagus 7·2; broad bean 6·92; *Spirogyra* 6·73 (at night); with indicators pH 7—6·5.
2. Plant cells with an acid reaction —
   pressed juice and H-electrode — unripe apples 4·5; unripe gooseberries 3·27; rhubarb leaves and petiole 3·09; white carnation, epidermis of flower 4·9—5·9; white tulip, epidermis of flower 5·2—5·5; with indicators pH 3·09—5·5.
3. Plant cells with acid or neutral reaction —
   this group includes a series of white flowers e. g. hyacinth, narcissus, *Magnolia Yulan*, *Trillium grandiflorum*, "*Mai-*

glöckchen", ox-eye daisy, true chamomile, dog chamomile, snowdrop, crocus, cherry, apple, pear. The epidermis was found by ROHDE's section-immersion method to be usually slightly alkaline or neutral, small-celled sub-epidermal parenchyma with strongly acid stripes, acid vascular strands, and between the acid stripes a large-celled parenchyma with a reaction similar to that of the epidermis.

4. A series of observations on indicator anthocyans e. g. beetroot, red cabbage, bilberry etc., and a series of observations on the behaviour with acid and basic dyes of naturally neutral and acid tissues in buffered solutions of lower or higher pH respectively.

The pH data for tissue reactions recorded by ATKINS (1922), using a similar immersion method and various indicators, including the very useful di-ethyl red, may be summarised thus .

Salvia verbenacea —	sclerenchyma and bast fibres	5·2	5·4
	wood walls	5·4	5·6
	medullary rays and parenchyma		6·0
Cochlearia armoracia —	sclerenchyma and vasc. bundles		4·6
	parenchyma of stem		6·0
	parenchyma of leaf		5·4
Taraxacum officinale —	stem		4·6
	leaf-parenchyma		5·8
	midrib		4·6
	rootstock-medulla		5·4
	latex canals	4·4	4·8
	"Vascular bundles much the same, but not quite as acid as the latex, which gave a more purple tint with methyl red."		
Anagallis arvensis —	stem-vascular bundles		5·2
	pith		5·2

SAMUEL (1927) records leaf mesophyll of *Prunus laurocerasus* as pH 5·6—6·0, usually pH 5·7—5·8; lamina uniform for any particular leaf; micro-hydroquinone method.

PFEIFFER (1925a) devised another section-immersion method which eliminated the use of two-colour indicators and also of standard buffered comparisons, and later (1927) published a series of pH values for the abscission layer of leaves after frost-fall. *Fuchsia* spp. 4·8—5·2, *Begonia* spp. 3·9—4·3, *Azalea* 6·2, *Aesculus* spp. 5·4—5·8, *Echeveria* spp. 6·2—6·8, *Cotyledon* 6·9—7·1.

Pfeiffer (1925b) also investigated the internal pH of plant tissues from quite a different point of view, considering in detail the relation of the form of calcium oxalate crystals to the pH of the medium in which they are formed.

According to **Pfeiffer** (1925b, pp. 66—67) we have an equilibrium of various oxalate forms thus —

$$CaC_2O_4 + H_2C_2O_4 \rightleftharpoons Ca(HC_2O_4)_2$$

with a dissociation constant

$$K_1 = \frac{[CaC_2O_4]\ [H_2C_2O_4]}{[Ca^{++}]\ [HC_2O_4^-]_2} \quad . \ . \ . \ . \ . \ . \ . \ . \quad (1)$$

and also $H_2C_2O_4 \rightleftharpoons H^+ HC_2O_4^-$

with a dissociation constant

$$K_2 = \frac{[H_2C_2O_4]}{[H^+][HC_2O_4^-]} \ \therefore \ K_2[H^+] = \frac{[H_2C_2O_4]}{[HC_2O_4^-]} \quad (2)$$

Now if we take λ as that part of the solubility of CaC_2O_4 which is independent of $[H^+]$, and the total solubility as $\Lambda = \lambda + [Ca^{++}]$ we have in (1) $Ca\,C_2O_4 = \lambda$ and substituting this value and also (2) in (1) we have

$$K_1 = \frac{\lambda K_2[H^+]}{[Ca^{++}][HC_2O_4^-]}$$

and transferring $[Ca^{++}]$

$$\text{we have } [Ca^{++}] = \frac{\lambda K_2}{K_1} \cdot \frac{[H^+]}{[HC_2O_4^-]} \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad (3)$$

$$\text{then } \Lambda = \lambda + \frac{\lambda K_2}{K_1} \cdot \frac{[H^+]}{[HC_2O_4^-]} \quad . \ . \ . \ . \ . \ . \ . \ . \quad (4)$$

Brinkman and **van Dam** (cited **Pfeiffer**) experimentally found the „Loslichkeitsprodukt" $[Ca^{++}]\ [C_2O_4^{--}]$ constant at ·555 millimols per litre at 20° C.

We can, therefore, take λ, K_1 and K_2 in (4) as constants and the total Ca^{++} in solution as oxalate is then seen to be determined by $[H^+]$ and $[HC_2O_4^-]$ in the form of the ratio $\dfrac{[H^+]}{[HC_2O_4^-]}$.

We can really go further than **Pfeiffer**, since the form of the fundamental equation for the oxalate or HAH type of acid is

$$K_1 = \frac{[H^+][AH^-]}{[HAH]} \ \therefore \ \frac{1}{[H^+]} = \frac{[AH^-]}{K_1[HAH]}$$

and therefore where the total concentration of A in all forms is constant (as well as K_1) the concentration $[AH^-]$ is governed

Tabellarisch seien (auszugsweise) einige Resultate der an-

Reagentien	pH
Experiment 1: Zusatz von	
konzentrierte $H_2C_2O_4$ + konzentrierte $CaNO_3$	4,4
konzentrierte $H_2C_2O_4$ + verdünnte $CaNO_3$	5,8
verdünnte $H_2C_2O_4$ + verdünnte $CaNO_3$	6,4
verdünnte $H_2C_2O_4$ + stärker verdünnte $CaNO_3$	7,0
stärker verdünnte $H_2C_2O_4$ + konzentrierte $CaNO_3$	7,6
stärker verdünnte $H_2C_2O_4$ + verdünnte $CaNO_3$	8,4
Experiment 2: Zusatz von	
konzentrierte $H_2C_2O_4$ + verdünnte $CaCl_2$ + Spur HCl	3,2
konzentrierte $H_2C_2O_4$ + verdünnte $CaCl_2$ + Spur HCH_3CO_2 . .	4,2
konzentrierte $H_2C_2O_4$ + fast konzentrierte $CaCl_2$	4,6
verdünnte $H_2C_2O_4$ + schwächer verdünnte $CaCl_2$	5,2
verdünnte $H_2C_2O_4$ + stärker verdünnte $CaCl_2$	6,0
verdünnte $H_2C_2O_4$ + stärker verdünnte $CaCl_2$	7,2
verdünnte $H_2C_2O_4$ + stärker verdünnte $CaCl_2$	7,8
verdünnte $H_2C_2O_4$ + verdünnte $CaCl_2$ + Spur HNO_3	8,2
Experiment 3: Zusatz von	
konzentrierte $H_2C_2O_4$ + konzentrierte $CaSO_4$	3,6
konzentrierte $H_2C_2O_4$ + schwächer verdünnte $CaSO_4$	4,2
verdünnte $H_2C_2O_4$ + verdünnte $CaSO_4$ + Spur HCl	4,8
konzentrierte $H_2C_2O_4$ + stärker verdünnte $CaSO_4$.	5,8
verdünnte $H_2C_2O_4$ + schwächer verdünnte $CaSO_4$.	6,8
verdünnte $H_2C_2O_4$ + stärker verdünnte $CaSO_4$	7,4
Experiment 4: Zusatz von	
konzentrierte $H_2C_2O_4$ + konzentrierte $(C_{12}H_{22}O_{11}\ CaO)$	5,2
schwächer verdünnte $H_2C_2O_4$ + konzentrierte $(C_{12}H_{22}O_{11}\ CaO)$.	5,8
konzentrierte $H_2C_2O_4$ + verdünnte $(C_{12}H_{22}O_{11}\ CaO)$	6,4
schwächer verdünnte $H_2C_2O_4$ + verdünnte $(C_{12}H_{22}O_{11}\ (CaO)$ . .	6,8
stärker verdünnte $H_2C_2O_4$ + verdünnte $(C_{12}H_{22}O_{11}\ (CaO)$ . . .	7,2
stärker verdünnte $H_2C_2O_4$ + stärker verdünnte $(C_{12}H_{22}O_{11}\ (CaO)$.	7,6
stärker verdünnte $H_2C_2O_4$ + stärker verdünnte $(C_{12}H_{22}O_{11}\ (CaO)$ + Spur HNO_3	8,2

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1) Calciummonosaccharat (Liquor calcis saccharatus) erhält man

Abfiltrieren der Lösung

gestellten organischen Experimente wiedergegeben:

% Kristalle			
monoklin	tetragonal	drusig	
Calciumnitrat zur Oxalsäure			
98	2	—	monokline Kristalle von erheblicher Größe
88	12	—	weniger große monokline Kristalle
48	52	—	—
5	87	8	kleine tetragonale Kristalle
—	94	6	—
—	97	3	große tetragonale Kristalle
Calciumchlorid zur Oxalsäure			
99	1	—	monokline Kristalle sehr groß
98	2	—	monokline Kristalle sehr groß
95	5	—	monokline Kristalle kleiner
76	24	—	tetragonale Kristalle groß, monokline klein
44	48	8	ähnlich wie vorige
2	76	22	tetragonale Kristalle ziemlich klein
—	84	16	—
—	78	22	—
Calciumsulfat zur Oxalsäure			
99	1	—	monokline Kristalle mittelgroß
96	4	—	monokline Kristalle groß
94	6		
78	22		tetragonale Kristalle mittelgroß
54	36	10	tetragonale Kristalle groß
12	82	6	tetragonale Kristalle mittelgroß
Calciummonosaccharat[1]) zur Oxalsäure			
72	28		
68	32		monokline Kristalle ziemlich groß
44	52	4	
26	58	16	Drusen sehr klein
5	84	11	monokline Kristalle überaus klein
—	91	9	tetragonale Kristalle groß
—	96	4	tetragonale Kristalle mittelgroß

durch Digerieren von 1 vol. $Ca(OH)_2$, 2 vol. $C_{12}H_{22}O_{11}$ and 20 vol. H_2O und nach einigen Stunden.

by $\frac{1}{[H^+]}$. Therefore we can say that the variable factor (at constant temperature) which governs the total solubility of a fixed concentration of calcium as oxalate is $[H^+]$.

Pfeiffer (ibid. p. 71) proceeds to discuss the relation of $[H^+]$ to the crystal forms of calcium oxalate and finds that the percentage of the various crystal forms varies with the pH and with the medium. As this method promises to be of some considerable usefulness in tissue researches the tabular statement of *in vitro* results is extracted from Pfeiffer's paper (see pp. 100 101).

These results show that whatever medium be used there is a large percentage of *large* monoclinic crystals, with very few *small* tetragonal crystals and no drusy crystals at all, formed below pH 5·0 (red with M.R.); while the percentage and size of monoclinic forms decrease, both percentage and size of tetragonal crystals increase with increase of pH beyond 5·0, drusy crystals appearing in small proportion only at or above pH 6·0. Above pH 7·0 practically all the crystals are tetragonal or drusy. The monoclinic forms include raphides and the sandy crystals of Rubiaceae and Solanaceae; while the tetragonal forms include the octohedral types of crystals (Czapek III p. 68).

Temperature changes within the plant being slight, this influence in the natural crystallisation of calcium oxalate in plants may be discounted. Then sphaero-aggregates are seen to indicate a pH of 6·0 or over; large monoclinic crystals with no tetragonal forms a pH of 5·0 or under; large tetragonal crystals with or without sphaero-aggregates with no monoclinic forms a pH of 7·0 or over. Pfeiffer discusses the action of diffusion on the total concentration of calcium and concludes that the zonal distribution of calcium oxalate crystals in plant periderm (secondary cortex) is due, not to a zoning of pH but to the Liesegang ring phenomena, which controls the total concentration of calcium. He gives no new data for definite plants but suggests in his summary that "In the secondary cortex of plants the reaction shows a maximum on the acid (pH 2·4—3·8) and another on the basic (pH 7·2—8·6 ?) flank of the protein iso-electric point", and that. "The actual acidity of the cells of the secondary cortex, especially during its development, ... is continually changing".

7. R.I.M. GENERAL SURVEY

In addition to these somewhat scattered observations there is the series of observations made by means of the R.I.M. by Rea and Small (1926). In that series some one hundred and sixty-four stems were examined, mostly flowering stems and mostly of different species. Sections were taken from the upper, middle and lower portions of each stem. The records for each portion of each stem were kept in detail but analysis of the results shows no very significant differences correlated with the portion sectioned. For example, the reaction was the same for all three portions in 102 cases of epidermis, 91 of cortex, 79 of endodermis, 88 of pericycle, 89 of phloem, 156 of xylem, 65 of pith in the ray region, and 77 of central pith. Further, taking all figures below pH 5·3 as acid and all figures above pH 5·5 as alkaline, the analyses show no very significant variation so far as upper, middle and lower portions are concerned, see Table V from which indeterminate ranges are omitted.

Table V

Tissue	Alkaline			Acid		
	Upper	Middle	Lower	Upper	Middle	Lower
Epidermis	20	31	26	115	109	103
Sub-epidermis	56	57	56	84	78	62
Cortex	75	80	74	64	59	44
Endodermis	56	64	63	78	62	61
Pericycle	28	32	21	75	83	106
Phloem	38	47	49	98	86	80
Xylem	1	3	3	160	160	159
Pith, ray	64	68	60	63	59	72
Pith, central.	58	49	57	53	16	33

This investigation has since been extended by Rea and Small to 193 species and 203 stems, and *the following is the first publication of the results*, which are combined here with such of the first series as were not revised. As before, since there is no important difference shown by the three portions of the stems used, the data for these portions have been combined in the published record, the indeterminate ranges being omitted. By the use of benzene-azo-α-naphthylanime (BAN) and bromo-cresol green

Table VI.

	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		Notes.
Triglochin palustre.	Zb	Zb	b	b	Z	Z	Z	—	—		flrg. stem
Alisma plantago	ca	ca	ca	ca	e(hk)	e(h)	ca1)	ce	ca		1) unlign.
Lilium trigrinum.	Z	Zb	Zb	Zb	Z	Z	Z	Zb	Zb		flrg. stem
Lilium tigrinum	b	b	b	b	Z	b	Z	b	b		veg. stem
Scilla non-scripta	c	c	c	c	Z	cZ	Z	c	c	(cult.)	flrg. stem
Scilla non-scripta	b	b	b	b	Z	b	Z	b	b	(wild)	do.
Convallaria majalis.	b	b	b	b	Z	Zb	Z	—	—		do.
	UE	PT	ST	Xy	Phl	Fbs	GC	LE			
Aloe variegata.	c	c	ce	g	c	—	c	c			leaf 'S' = succul.
Haworthia reinhardtia . . .	e	e	e	g	e	gkgg	e	e		cutic. g	leaf 'S'
Gasteria verrucosa	acc	c	c	g	c	—	acc	acc		cutic. k	do.
	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		
Tradescantia virginica . . .	ce	ce	a	a	c(h)	c	h	a	b	coll. e	flrg. stem.
	UE	PT	ST	Xy	Phl	Fbs	BS	GC	LE		
Puya sp.	i	i	i	i	ig	ik	i	—	i	cutic. k glands a	leaf 'S'
	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		
Orchis maculata	Z	Z	Z	Z	Z	Z	Z	Z	Z		flrg. stem
Orchis mascula	b	b	b	b	Z	b	Z	bc	bc		do.
Listera ovata	Zb	Zb	—	b	Z	Z	Z	—	—		do.

Fagus sylvatica	Z	Z	b	bZ	Z	Z	Z	b	b	(hypocotyl)	
Urtica dioica	b	b	Zb	Zb	Z	Zb	Z	bZ	b		(yg. veg. stem)
Rumex acetosa	Z	Z	Z	Z	Z	Z	Z	Zb	bZ		flrg. stem
R. acetosella	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
R. crispus	e	h	h	h	hk	h	hk	eh	eh		do.
R. obtusifolius	e	eec	eec	e	eh(k)	e	hhk	ec	ec	coll. h.	do.
Polygonum amphibium	e	eeh	e(h)	eh	h	h	h	ehk	e	glands h.	do.
P. aviculare	hhe	hhe	hhe	hhe	h hk	hhe	h hk	e eh	e ec		do.
P. persicaria	e	e eh	e eh	e eh	h	h	h	e eh	eceh		do.
Beta maritima	Z	bZ	bZ	Zb	Zb	Z	Z	b	b		do.
Chenopodium album	Z	Z	Z	Z	—	—	Z	Z	Z		do.
Atriplex angustifolia	c	c	c	ab(Z)	—	c	Z	b(Z)	b		do.
A. sp.	Z	c	c	c	Z	—	Z	c	c		veg. stem
Salsola kali	Z	b	b	b	—	bZ	Z	b	b		'S'
	UE	PT	ST	Xy	Phl	Fbs	BS	GC	LE		
Mesembryantheum tigrinum	e	e	e	g	e	e	e	e	e	prickle e	leaf 'S'
M. stelligerum	ie	ie	ie	g	eg	—	—	ie	ie	hairs e	do.
	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		
Stellaria media	c(a)	a	a	aac	aac	a	h	a	—	hairs c	flrg. stem
S. holostea	Zc	Z	Z	Zc	cZ	cZ	Z	Zc	—		do.
Cerastium tomentosum	a(e)	a(e)	a(e)	a	a(e)	a	h	a	a	hairs e	do.
C. triviale	c	a	a	ac	a(h)	a	h	a	—	hairs e	do.
Arenaria peploides	a	a	a	acc	hk	c	h	aah	a	cutic. e	do.

A 6·2, a 5·9, b 5·9—5·6, c 5·6, d 5·6—4·8, e 5·2—4·8, f 5·2—4·4, g 4·4, h 4·4—4·0, i 4·0, k <3·4, Z 5·2—4·0.

	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		Notes
Silene maritima	cca	ca	caa	caa	ehk	ca	h	(c)a	a		flrg. stem
Dianthus caryophyllus . . .	cac	caa	caa	cca	c hk	cac	h	a	a	med. hbtt.	do.
	cac	a	a	a	a hk	a	h	a ea	a	damp. hbtt.	do.
Lychnis sp.	—	b	b	b	Z	Z	Z	—	—		yg. flrg. stem
	c	bc	cb	c	Zc	c	Z	b	—		veg. stem in sun
	Z	Zb	Zb	Z	Z	Z	Z	Zb	—		veg. stem in shade
Ranunculus acris	c	c	aac	aac	a(hk)	c	h	a(ec)	a	hairs e	flrg. stem
R. auricomis	Z	b	b	b	b	b	Z	b	—		do.
R. bulbosus	Zb	—	Z	b	Zb	bZ	Z	b	b		do.
R. flammula	Z	b	b	—	Z	Z	Z	Z	—		do.
R. repens	c	a	a	a	a(h)	ac	h	a	a	hairs e	do.
Trollius europaeus	Z	bZ	Z	Z	Z	Z	Z	Z	Z		flrg. stem
Delphinium ajacis	Zb	Zb	b	b	bZ	Zb	Z	b	—		do.
Anemone japonica	a	a	a	a	(a)hk	a	h	a	a	hairs e	do.
Anemone japonica	c	c	c	c	(c)hk	c	h	a	a		do.
Aconitum napellus	caa	caa	a	a	(a)hk	a	h	a	a	hairs e	do.
Aconitum napellus	c	c	$a^1 c^2$	chw	(c)hk	a	h	a	a	cuticle e	a^1 outer c^2 inner
Aconitum napellus	b	h	b	b	Z	b	Z	b	h		yg. veg. stem
Papaver rhoeas	b	bZ	b	b	b	h	Z	b	h	large hairs	flrg. stem
Papaver (Shirley)	ae	ae	ae	ae	a(hk)	ac	h	a	a	k	do.
P. somniferum	a(h)	a(h)	a	a	a	a	h	a	a		do.
Fumaria officinalis	Zb	Zb	Zb	bZ	Z	Z	Z	b	b		do.
Dicentra formosa	ac	ac	(a)ch	(a)ch	a(ch)	a	h	aca	aca		do.

Dicentra formosa	c	c	ch	ch	h	a	h	a	a		do.
Matthiola incana. . . .	—	b	b	b	—	b	Z	Z	—		do.
Cheiranthus cheiri	b	b	b	—	—	b	Z	Zb	Z		do.
Nasturtium officinale. . . .	Z	Z	Z	Z	—	cZ	Z	c	c		do.
Barbarea vulgaris	bZ	bZ	Zb	b	bZ	Zb	Z	bZ	b		do.
Arabis sp.	Z	—	b	bZ	Z	b	Z	Z	—		veg. stem
Cardamine pratensis	b	b	b	b	b	b	Z	bZ	b		flrg. stem
Brassica campestris	Z	Z	Zb	—	Z	Z	Z	Z	—		do.
Iberis amara	—	b	b	b	—	—	Z	Zb	b		do.
Capsella bursa-pastoris . . .	c	a	a	a	a	a	h	a(h)	a	glands a.	do.
Cakile maritima	Zb	Zb	b	Z	Z	Z	Z	Z	Z		
Reseda luteola	Z	Z	Z	Zc	Z	Z	Z	Z	Z		do.
R. odorata	Z	bZ	bZ	bZ	Zb	b	Z	b	b		do.
R. suffruticulosa.	c	c	a	a	—	a(h)	hk	a	a	cuticle e hairs e	do.
Echeveria metallica	Z	Z	Z	Z	Z	Z	Z	Z	Z		'S' do.
Crassula lycopodioides . . .	e	e	e	e	—	e	g	e	e		'S' veg. stem
C. sp.	ei	i(e)	ie	e	e	e	g	e	e		'S' yg. veg.stem
Sedum praealtum	e	e	e	e	e	e	g	e	e		'S' veg. stem
	UE	PT	ST	Xy	Phl	GC	LE				
Sedum praealtum	ce	c	c	g	eg	e	e			leaf — daylight	'S'
Sedum praealtum	ec	e	e	g	e	ec	ec			leaf — 20 hours in dark	
Sedum sp.	e	e	e	g	e	e	e			leaf.	'S'
Crassula lycopodioides . . .	e	e	e	g	e	e	e			do.	'S'

A : 6·2, a 5·9, b 5·8—5·6, c 5·6, d 5·6—4·8, e 5·2—4·8, f 5·2—4·4, g 4·4, h 4·4—4·0, i 4·0, k <3·4, Z 5·2—4·0.

	UE	PT	ST	Xy	Phl	GC	LE				Notes
Crassula portulaca	i	e	e	g	e	e	e			leaf.	'S'
C. rosea	i	ie	ie	g	e	i	i			do.	'S'
C. sp.	e	ig	ig	g	e	e	e			do.	'S'
Bryophyllum calycinum	e	e	e	g	e	e	e			do.	'S'
Echeveria glauca	i	e	e	g	e	e	e			do.	'S'
E. metallica	ie	e	e(c)	g	e	e	ie			do.	'S'
	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		
Saxifraga umbrosa	Z	Z	Z	Z	Z	Z	Z	Z	Z		
Tellima grandiflora	Z	Z	Z	Z	Z	Z	Z	Z	Z		
Ribes grossularia	Z	Z	Z	Z	Z	Z	Z	Zc	cZ		
R. sanguineum	Z	Z	Z	Z	Z	Z	Z	Z	Z		
Potentilla palustris	Z	Z	Z	Z	Z	Z	Z	Z	Z		flrg. stem
Geum rivale	Z	Z	Zb	Z	Z	Z	Z	Z	Z		do.
Geum sp. (cult.)	h	h	c	c	h	h	h	c	c	hairs he	do.
Geum sp. (cult.)	h	h	e	e	h(k)	h	h	ec	ec	hairs e	do.
Ulmaria palustris	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
Alchemilla vulgaris	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
Alchemilla vulgaris	cZ	c	Zc	Z	Zc	Z	Z	Z	c		do. (younger)
Lupinus albus	Z	Z	c	Z	Z	Z	Z	c	c		flrg. stem
Trifolium pratense	ca	ca	ca	ca	ch	ca	h	a	a	hairs c	do.
T. repens	Z	Z	Zb	Zb	Z	Z	Z	Zb	bZ		do.
Vicia faba	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.

Vicia faba	b(AZ)	bZ	b	b	k	—	Z	b	—		veg. stem
Vicia sepium	Zb	b	b	b	bZ	Ab	Z	b	b		flrg. stem
Pisum sativum	Z	b	b	b	Z	bZ	Z	b	b		do.
Lathyrus macrorrhizus	Z	—	—	—	Z	Z	Z	bZ	b		do.
Geranium dissectum	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
G. robertianum	eh	eh	e	e	e(hk)	e(hk)	h	ec	ec	hairs e	do.
G. sylvaticum	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
G. sp. cult.	e	e	e	ec	e	e	h	e(a)	e(a)	hairs ca	do.
G. sp. cult.	h	h	c	c	e(hk)	h	h	e(h)	e(h)	hairs ea	do.
Erodium cicutarium	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
Pelargonium sp. (cult.)	h	h	h	h	hk	h	h	(c)h	(c)h	hairs e	do.
Pelargonium sp. (cult.)	e	e	e	e	hk	e	h	(e)c	(e)c	hairs e	do.
Tropaeolum majus	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
Tropaeolum majus	ec	ec	c	c(h)	—	c	h	caa	caa		veg. stem
Limnanthes douglasii	b	b	b	b	b	b	Z	b	b		flrg. stem
Limnanthes douglasii	a	a	a(c)	a(c)	a	a(c)	h	a	a(c)		do.
Polygala vulgaris	Z	—	b	b	—	b	Z	b	—		do.
Ampelopsis hederacea	Z	Z	Z	Z	Z	Z	Z	Z	Z		veg. stem
Euphorbia helioscopia	Z	Z	—	—	—	—	Z	—	—		flrg. stem
Euphorbia globosa	e	e	e	e	e	e	g	e	e	cutic. g	'S' veg. stem
Mercurialis annua	Z	Z	Z	b	—	Z	Z	b	b		flrg. stem
Acer pseudoplatanus	Z	Z	Zb	Z	bZ	Z	Z	bZ	bZ		hypocotyl
Hypericum quadrangulum	h	h	h	e	h	h	hk	e	e		flrg. stem
Epilobium montanum	nat. pink	h	ec	hc	—	e	hk	e	ec	hairs c	veg. stem

A > 6·2, a 5·9, b 5·9—5·6, c 5·6, d 5·6—4·8, e 5·2—4·8, f 5·2—4·4, g 4·4, h 4·4—4·0, i 4·0, k < 3·4, Z 5·2—4·0.

	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		Notes
Epilobium montanum . . .	nat. pink.	c	c	c	c	c	h	c	c	hairs c	flrg. stem
Epilobium montanum . . .	do.	h	c	c	—	h	h	he	c	hairs e	do.
Clarkia elegans	Z	Z	bZ	bZ	—	Z	Z	b	b		do.
Viola cornuta	c	cca	a	a	(a)ch	caa	h	a	—		cult.
Cereus flagelliformis	ie	e	eie	e	e	g	g	e	e	branches k branches k	veg. stem 'S'
Phyllocactus sp.	e	e	e	(e)ik	e	(g)ik	e	e		cuticle e cuticle i	'S' veg. stem
Echinocactus ingens	i	e	e	—	—	e	g	e	e	spine k	do.
Hedera helix	h	h	h	h	b	b	b	b	b		hypocotyl Xy unlign.
Anthriscus sylvestris	Z	Zb	Zb	Zb	—	bZ	Z	b	b		flrg. stem
Apium graveolens	c	c	c	—	c	c	Zc1)	Zc	—	1) parench.	do.
Apium graveolens	Z	—	—	Z	Z	Z	Z	Z	Z		younger
Carum petroselinum	Z	b	b	b	b	Z	Z	Z	—		flrg. stem
Aegopodium podagraria . .	Z	—	b	bZ	Z	Z	Z	Zb	—		do.
Oenanthe crocata	Z	Z	b	b	Z	Z	Z	Ab	Ab		do.
Aethusa cynapium	Z	Z	Z	b	—	Z	Z	Zb	b		do.
Heracleum sphondylium . .	a	a	a	a	a	ac	h	a(ck)	a	hairs ec	do.
Daucus carota.	h	h	ea	a	—	a	h	a(ch)	a	hairs e	do.
Primula vulgaris.	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
P. auricula	bZ	b	b	b	Z	b	Z	b	b		do.
Lysimachia nemorum . . .	Z	Z	Zb	Z	Z	Z	Z	Zb	bZ		veg. stem

Armeria maritima	ec	ec	ec	ec	c(hk)	c	h	c	c		flrg. stem
Armeria maritima	e	e	e	e	e(hk)	eh	h	he	c	hairs e	do.
Ligustrum vulgare	—	—	b	bZ	bZ	b	Z	bZ	bZ		veg. stem
Vinca major.	Z	Z	Z	bZ	Zb	bZ	Z	b	b		do.
Calystegia sepium	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
Phlox sp. cult.	ac	ac	a	a(e)	a(hk)	a	hk	a(e)	a		flrg. stem
Symphytum officinale . . .	Z	Z	b	bZ	bZ	Zb	Z	b	b		flrg. stem
Borago officinalis	Z	Z	bcZ	—	Z	Z	Z	Zb	Z		do.
Myosotis palustris	c	c	ec	c	c(hk)	c	h	eaa	a	hairs c	do.
Myosotis palustris	c	a	ah[1])	caa	c(hk)	c	h	he	a	do. [1]) spots	do.
Buddleia variabilis	Zc	ab	b	Zc	Z	Z	Z	b	ab		do.
Ajuga reptans.	—	—	b	b	—	b	Z	Zb	—		veg. stem
Teucrium scorodonia	Z	b	b	Ab(Z)	Z	b	Z(A)	Ab	Ab		do.
Scutellaria galericulata . . .	a	a	a	a	a(hk)	a	hk	a	a	hairs c	do.
Scutellaria galericulata . . .	Z	Z	Z	Z	Z	Z	Z	Z	Z		flrg. stem
Stachys palustris	c	c	c	c	e(hk)	ec	hk	a(hk)	c	hairs e	do.
S. sylvatica	a	a	a	a	a(hk)	a	h	a(hk)	a	hairs eh	do.
Prunella vulgaris	—	c	a	cch	cch	c	h	c(eh)	c		do.
Prunella vulgaris	—	c	cac	cce	c	c	h	c	cac	hairs c	do.
Lamium album	c	c	c	c	c	c	h	c	c	hairs e & c	do.
L. purpureum	—	a	a	a	a	a	h	a	a	hairs e	do.
L. purpureum	—	bZ	b	bZ	b	b	Z	b	b		veg. stem
Salvia officinalis.	ca	ca	ca	ca	h	ca	h	c	c	hairs c	do.
Salvia officinalis.	Z	Z	Z	Z	Z	Z	Z	Z	Z		flrg. stem
Mentha viridis.	Z	Z	Z	Z	Z	Z	Z	Z	—		do.

A 6·2, a 5·9, b 5·9—5·6, c 5·6, d 5·6—4·8, e 5·2—4·8, f 5·2—4·4, g 4·4, h 4·4—4·0, i 4·0, k 3·4, Z 5·2—4·0.

	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		Notes
Solanum dulcamara	e	nat. pink	a	a	a	a(h)	h	a	a	hairs e & A	veg. stem
Solanum dulcamara	Z	Z	Z	Z	Z	Z	Z	Z	Z		flrg. stem
S. nigrum.	Z	b	ab	b	—	Z	Z	Zb	Zb		veg. stem
S. lycopersicum	a	a	a	a	a	a	h	a	a	hairs e & c	do.
S. tuberosum	a	a	a	a	a	a	h	a	a	hairs e & A	do.
S. tuberosum	ec	ac	a(c)	caa	chh	caa	h	ahh	a	hairs e & A	do.
Nicotiana tabacum.	b	—	b	—	b	b	Z	b	b		do.
Antirrhinum majus	c	caa	caa	a	a	ca	h	a	a	hairs e	veg. stem
Antirrhinum majus	Zb	Zb	Zb	b	Z	Z	Z	bZ	b		flrg. stem
Scrophularia nodosa	a	a	a	c	hk	a	hk	ec	ac		veg. stem
Scrophuloria nodosa	Z	Z	Z	Ab(Z)	Z	Z	Z(A)	b	b		flrg. stem
Mimulus luteus	—	b	b	Zb	b	b	Z	Zb	b		do.
M. moschatus	a	a	a	a	a	a	h	a	a		veg. stem
M. moschatus	Z	Z	c	Zc	Z	Zc	Z	cZ	c		do.
Veronica beccabunga. . . .	c	c	c	bZ	—	Zb	h	caa	—		'S' veg. stem
V. chamaedrys	Z	Z	—	Z	Z	Zb	Z	Z	Z		flrg. stem
Digitalis purpurea	Z	Z	b	—	Z	Z	Z	b	b		do.
D. sp. (white cult.)	c	ca	ca	ca	ca(hk)	ea	h	a	a	hairs e	do.
Pedicularis palustris	b	—	—	bZ	Z	Z	Z	b	b		do.
Calceolaria sp..	Z	Z	Z	Z	—	Z	Z	(a)Z	(a)Z		veg. stem
Plantago lanceolata	ece	ece	a	a	h	h	h	a(ec)	a	hairs h	flrg. stem
Plantago lanceolata	c	c	a	a	ch	c	h	a	a		do.
Plantago major	Zb	c	c	c	ch	c	h	b	b		do. (older)

Plantago major	Z	Zb	Zb	Z	Z	Z	Z	Z	Z		do. (younger)
Sherardia arvensis	Z	Z	Zb	Z	Z	Z	Z	Z	Z		flrg. stem
Asperula odorata	Z	b	b	bZ	b	Zb	Z	bZ	bZ		veg. stem
Galium aparine	a	a	a	a	—	a	h	a	a	hairs e	do.
G. verum	e	e	e	e	—	eec	hk	c	c	hairs e	do.
Lonicera periclymenum	Z	—	—	—	Z	Z	Z	bZ	b		
Valerianella olitoria	Zb	Zb	b	b	b	b	Z	b	b		veg. stem
Centranthus ruber	a(e)	a(e)	ac	a	a	a	h	a	a		flrg. stem
Scabiosa arvensis	e	e	ac	ac	ac	ac	h(k)	(a)ch	a	hairs e	do.
S. succisa	e	e	ca	c	c	c	hk	ch_w	a	h_w = walls	do.
Campanula rotundifolia	Z	b	b	Zb	—	Z	Z	b	b		do.
C. sp. (cult.)	e	e	ec	c	cch	c	h	c(ec)	ca	dry	do.
C. sp. (cult.)	c	a	a	a	a	cca	h	ahh	a	damp	do.
Agrimonia eupatoria	e(hk)	e(hk)	ec	cee	c	c	h	ec	ec	hairs c glands e	do. do.
Aster tripolium	eb	eb	a	a	hk	a	h	bh	a		do.
A. sp. (cult.)	ce	ca	ca	ce	ch	c	h	a	a	hairs e & c	do.
Solidago sp.	e	e	ec	c	ce	c	h	a	a	hairs e	do.
Bellis perennis	Zb	ca	b	b	ah	ac	h	b(Z)	a	hairs a	veg. stem
Helianthus tuberosus	h	e	a	a	a	ca	hk	a	a	hairs c & A	flrg. stem
Spilanthes sp.	e(hk)	e(hk)	c(k)	c	hk	c	h	b	a	hairs c & A	do.
Inula helenium	h	h	ah	a	h	ch	h	ec	a	hairs e	do.
Achillea millefolium	e	e	c	c	(c)hk	c	h	ac	a	hairs e shady	do.
Achillea millefolium	h	h	e	ha	hk	ha	h	a(h)	a	hairs e dry	do.
Matricaria inodora	ca	ca	a	e	ch	hc	h	ah	a		

A . 6·2. a 5 9. b 5·9—5 6. c 5 6. d 5·6—4·8. e 5·2—4 8. f 5·2—4·4. g 4·4. h 4·4—4·0. i 4·0, k < 3·4, Z 5·2—4·0.

	Ep	Sub	Co	En	Pe	Phl	Xy	PR	PC		Notes
Matricaria inodora	eh	eh	a	a	h	h(a)	h	a	a		flrg. stem
Chrysanthemum maximum	e	e	(c)a	(c)a	e(h)	e	h	eb	a		do.
C. segetum	ha	ca	ca	ca	hk	c	h	a(k)	a	Bot. Gdn.	do.
C. segetum	ha	ha	a	a	a(h)	a	h	a	a	seaside	do.
C. hort.	b	b	b	b	Z	b	Z	b	b		veg. stem
Tanacetum vulgare	Z	Z	Z	Z	Z	Z	Z	Zb	Zb		flrg. stem
Artemisia sp.	—	b	b	—	—	Z	Z	b	b		veg. stem
Doronicum pardalianches	ae	ae	a	a	a	ca	h	a	a	hairs c	yg. flr. bud.
Doronicum pardalianches	e	e	a	a	a(hk)	a	h	a(h)	a	hairs c	flrg. stem
Senecio jacobaea	c(e)	c(e)	a(e)	a	a(h)	a	hk	a(h)	a	hairs e cuticle e coll. e	do.
S. sylvaticus	c	c	ac_w	a	(a)hk	a	hk	a	a	hairs e c_w=walls	flrg. stem
S. vulgaris	b	b	b	b	—	b	Z	b	b		veg. stem
Kleinia articulata	e	e	e	e	—	e	g	e	e	petiole	'S' do.
Calendula officinalis	Z	—	—	—	—	Z	Z	—	—		flrg. stem
Cnicus lanceolatus	Z	Z	Z	Z	Z	Z	Z	Z	Z		do.
Centaurea montana	c	c	c	c	(e)hk	c	h	e	a	hairs c	do.
C. nigra	b	b	b	Z	Z	—	Z	Z	—		do.
Lapsana communis	c	ca	ca	ca	a	a	h	a(h)	a	cuticle e	do.
Crepis biennis	eh	eh	ca	ca	ca	ca	hk	bh	—	hairs e	do.
C. virens	hZ	Z	Z	hZ	Z	Z	Z	Zh	Zb		do.

Hieracium anglicum	Z	b	b	bZ	Z	Z	Z	Z	—		do.
H. aurantiacum	c	c	(c)a	(c)a	a	(c)a	h	ac	a	hairs e & c cutic. e	do.
H. murorum	Z	b	b	b	Z	Z	Z	Z	Zb		do.
H. pilosella	Zc	Zc	Zb	Zc	Z	Zc	Z	Zc	bZ		veg. stem
Hypochoeris radicata. . . .	c	c	a	a	a(hk)	a	h	a	—		flrg. stem
Hypochoeris radicata. . . .	e	e	ac	ac	c(hk)	c	h	a(h)	—	hairs e	do.
Taraxacum officinale. . . .	eh	eh	ca	ca	c(e)	c(e)	h	ca	c		do.
Sonchus oleraceus	a	a	a	a	a(h)	ac	h	a	a	hairs e coll. h	do.
	UE	PT	ST	Xy	Phl	GC	LE				
Kleinia articulata	e	e	e	g	e	e	e			leaf.	'S'

A 6·2, a 5·9, b 5·9—5·6, c 5·6, d 5·6—4·8, e 5·2—4·8, f 5·2—4·4, g 4·4, h 4·4—4·0, i 4·0, k <3·4, Z 5·2—4·0.

(BCG) the **Z** range pH 5·2—4·0 has been split up in many cases into **e** (pH 5·2—4·8), **g** (pH 4·4), **h** (pH 4·4—4·0) or **i** (pH 4·0). The ranges indicated by one letter refer to cases in which the three sections from each of the three portions either gave the same reaction in every section or, by reason of one or more of the indicators failing to give a *definite colour*, one or more of the sections gave a wide range while the others were definite and the same for each section. In the cases where more than one range (letter) is given there was a definite variation from one portion to another of the stem, or a definite variation from cell to cell in the same tissue in the same section. Where it could be determined definitely as confined to the wall, a different pH for that part of the cell is indicated by a letter with a small w added thus **hw**. This distinction, however, requires plasmolysis or other special treatment and the aim of this survey was to provide a general basis for the selection of detailed work on particular problems.

The so-called 'acid' range lies below pH 5·3. The so-called 'alkaline' range lies above pH 5·5. This division was, and still is, considered suitable for a general survey of plant tissue reactions in the Angiospermae. It differentiates the 'acid' families from the others as described below and may in the end prove more useful than a classification based upon the hypothetical iso-electric points of proteins in the living cell.

It should be noted that the ranges recorded refer in most tissues to the cell contents in general, with the exception of the xylem where the reaction of the walls of the vessels is recorded as a rule. The xylem parenchyma is usually in the same pH range as the ray region of the pith, but lignified walls in general (xylem, pericycle and bast fibres, etc.) were always found to be relatively acid. Cuticle and suberised walls were also in general 'acid'. Cellulose as a rule does not take up the colours, and it is usually possible to see the cell contents, except in small cells with suberised or lignified walls (e.g. endodermis, cork, sclereids). Further work on selected cells indicates that there may be a difference in the tint given by the nucleus, cytoplasm and sap, but in very few cases has a definite difference in the kind of colour been found, (see also Chapter XVI).

Table VII

Stem tissues only

	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	
Monocotyledons											
'acid'	2	1	1	1	9	3	9	1	1	—	below pH 5·3
'alkaline' . . .	5	4	7	8	—	4	1	4	5	—	above pH 5·5
Archichlamydeae											
'acid'	59	45	36	37	51	50	97	35	30	12	< 5·3
'alkaline' . . .	22	31	39	35	11	31	1	34	42	4	> 5·5
ditto - less 'all-acid' families											
'acid'	31	18	11	10	22	21	67	12	10	8	> 5·3
'alkaline' . . .	22	31	39	35	11	31	1	34	42	4	< 5·5
Sympetalae											
'acid'	29	23	9	12	30	21	82	10	7	19	5·3
'alkaline' . . .	22	34	49	38	15	39	-	30	53	10	< 5·5
Totals											
'acid'	90	69	46	50	90	74	188	46	38	31	5·3
'alkaline' . . .	49	69	95	81	37	74	2	68	100	14	5·5
ditto - less 'all acid' families (30 species)											
'acid'	62	42	21	22	61	45	158	23	18	27	5·3
'alkaline' . . .	49	60	95	81	37	74	2	68	100	14	5 5
Mixed or Varied											
Monocotyledons .	3	4	1	1	1	3		2	1		
Archichlamydeae	15	18	21	22	22	11	1	29	12	3	
Sympetalae . .	27	22	21	28	30	22	2	23	15	8	
Totals	45	44	43	51	53	39	3	74	28	11	

Analysis of Table VI

Epidermis. -- Amongst the Monocotyledons a definitely 'alkaline' stem epidermis occurs in *Alisma*; 2/3 Liliaceae, also in *Lilium tigrinum* (veg.); 1/3 Orchidaceae. Total 4/9.

Amongst the Archichlamydeae a definitely 'alkaline' stem epidermis occurs in *Urtica*; 1/5 Chenopodiaceae; 5/8 Caryophyllaceae, also in *Lychnis* sp. (veg.) in sun; 4/9 Ranunculaceae;

2/5 Papaveraceae; 3/10 Cruciferae; 1/3 *Reseda* species; 1/7 Leguminosae; *Limnanthes*, *Viola*, *Hedera*, 1/8 Umbelliferae, also in the older flowering stem of *Apium graveolens*. Total 23/99. In all other cases in the Archichlamydeae the epidermis of the flowering stem is either acid in all portions or at least in one, either upper middle or lower portion of the stem.

Amongst the Sympetalae a definitely 'alkaline' stem epidermis occurs in *Phlox*; 1/3 Boraginaceae; 3/10 Labiatae, also in *Scutellaria* and *Salvia* (veg.); 2/5 Solanaceae (mostly veg.); 2/10 Scrophulariaceae, also in *Antirrhinum*, *Scrophularia*, *Mimulus* (veg.); 2/4 Galieae; 2/2 *Scabiosa*; 8/34 Compositae, also in *Achillea* (shady) 1/2 specimens of *Matricaria* and *Hypochoeris*. Total 21/84.

It is clear, therefore, that on the whole the epidermis of the young flowering stem tends to be acid; with the vegetative stem of the same plant showing relative alkalinity in a number of cases (cp. Scrophulariaceae). The comparatively few monocotyledons examined show no definite tendency for the epidermis; the Liliaceae tending slightly to be alkaline and the Orchidaceae to be acid.

Sub-epidermis. — This tissue is usually very similar to the epidermis in its reaction and when there is variation it is towards the relatively alkaline reaction of the underlying cortex.

Cortex. — This tissue in the flowering stem is more frequently alkaline in the following groups (the figures give the ratio of species with definite alkalinity to those with definite acidity, indeterminate ranges being omitted): Liliaceae 2/1, Monocotyledons 6/1; Chenopodiaceae 3/1, Caryophyllaceae 5/1, *Ranunculus* 4/1, Ranunculaceae 7/2, Papaveraceae 2/0, Cruciferae 7/1, Leguminosae 4/1, Umbelliferae 5/1, Labiatae 7/3, Scrophulariaceae 3/2, Galieae 3/0. Valerianaceae 2/0, Dipsaceae 2/0, Compositae 19/3. Otherwise it is mixed or acid, if single representatives of families be omitted.

Endodermis. — This tissue is more frequently 'alkaline' in Liliaceae 4/1, Orchidaceae 2/1, Chenopodiaceae 2/1, Caryophyllaceae 6/0, *Ranunculus* 4/0, Ranunculaceae 6/1, Papaveraceae 2/0, Cruciferae 7/1, Leguminosae 3/1, Umbelliferae 5/1, Labiatae 6/3, *Solanum* 4/0 (veg.), Valerianaceae 2/0, Dipsaceae 2/0, Compositae 22/4.

Pericycle. — This is 'acid' in all the Monocotyledons examined and also in the large majority of the Dicotyledons. It is sometimes 'alkaline' in Labiatae, e. g. *Lamium*; also in *Solanum* 3/0 (veg.); Valerianaceae 2/0; Dipsaceae 2/0.

Phloem. — The contents of the sieve-tubes are more frequently 'alkaline' in the Caryophyllaceae 6/0, *Ranunculus* 3/1, Ranunculaceae 5/2, Papaveraceae 4/1, Cruciferae 5/2, Labiatae 6/3, Valerianaceae 4/1, Dipsaceae 2/0, Compositae 19/9.

Otherwise 'alkalinity' occurs only in scattered cases. The 'acidity' recorded in some cases is the virage given by the walls which may show 'acid' with 'alkaline' contents but in a large number of species a degree of acidity corresponding to pH 5·2—4·8 is found for the sieve-tube contents.

Xylem. — In all cases lignified walls in the xylem and elsewhere give an 'acid' virage indicating an acidity corresponding to pH 4·4—4·0 or even lower to 3·4. The truth of this virage as an expression of pH has been questioned, but the evidence in support of these colours being an indication of acid strength is more or less conclusive. When the lipoid error is considered (see p. 30), a definite colouration apart from the neutral tint of the indicator is seen to mean something. Neutral oils take up the neutral tint from both acid and alkaline aqueous indicator fluids; so that when we find the xylem walls showing a series of indications acid or alkaline according to the indicator used the virage can be taken as true. The series which show steadily acid, even to the lowest indicator have not the same evidential value, but it will be seen later that tissues, walls, which show this extreme acidity pass through a stage of lesser acidity during their development and maturation. The virage indicating the still greater acidity of mature walls would appear to be as true a measure of acidity as that of immature walls which show 'acid' to some indicators and 'alkaline' to others (see Chapter XVIII, for a discussion of the possible significance of these virages).

Another point of interest lies in the evidence here presented upon the generalisation by PRIESTLEY (1928) that cambial activity takes place where there is a pH gradient, as from acid xylem to alkaline phloem. From the above summary it will be seen that the xylem is almost uniformly around pH 4·4—4·0 or more acid and that this is a wall-acidity. A few records are given of 'alkaline' cell contents but these refer to the xylem parenchyma or to the unlignified vessel walls as in the hypocotyl of the ivy (q. v.). It will be seen also that the phloem is sometimes relatively alkaline, pH 5·9—5·6; that sometimes it is recorded as Z, in the wide range pH 5·2—4·0; and that when the Z range has been divided the phloem is frequently e (pH 5·2—4·8) while the xylem wall is g or h (pH. 4·4—4·0). The general pH gradient between phloem and xylem varies therefore from 1·5 to ·4 in extent. While the larger gradient might possibly have a physiological significance in relation to cambial activity, it seems doubtful whether the smaller gradient could have any such effect. This theory is, of course, based upon certain suppositions concerning the isoelectric points of proteins in living cells, and there is quite a large body of facts which indicate that the proteins of living cell have no definite isoelectric point at all. That this is so should be definitely taken into consideration in any application of the facts given in Table VI to the support of such speculations.

Pith, ray. — The medullary tissue in the ray region is more frequently 'alkaline' in the Chenopodiaceae 3/1, Caryophyllaceae 4/0, *Ranunculus* 3/1, Ranunculaceae 6/2, Papaveraceae 5/0, Leguminosae 4/0, Labiatae 3/1, Scrophulariaceae 5/1, Galieae 2/1, Valerianaceae 2/0, Compositae 12/5. Otherwise it is acid or varies from the upper to the lower portion of the stem, single representatives being omitted.

Pith, central. — The central medullary tissue is more frequently 'alkaline' in Chenopodiaceae 4/1, Caryophyllaceae 4/0, *Ranunculus* 3/0, Ranunculaceae 5/1, Papaveraceae 5/0, Cruciferae 5/2, Leguminosae 5/1, Umbelliferae 5/1, Boraginaceae 2/1, Labiatae 6/0, Solanaceae 4/0 (veg.), Scrophulariaceae 7/1, Galieae 2/1, Valerianaceae 2/0, Dipsaceae 2/0, Compositae 22/1. This tissue is seen to be very generally alkaline, except in the acid families enumerated below.

Hairs. — In the re-investigation of stem tissue reactions the hairs of a number of species were examined. These were more frequently of pH 5·2—4·8, sometimes of pH 4·4—4·0, giving 31 species with 'acid' hairs; in some cases the pH was higher 5·9—5·6 or 5·9 giving 14 species with 'alkaline' hairs. In 11 species hairs of two kinds 'acid' and 'alkaline' were found on the same stems.

SUMMARY OF THE REACTIONS OF FAMILIES

Apart from the tissues which are more normally 'acid', e. g. epidermis, pericycle and xylem, the tissue masses of the young flowering stem show a general alkaline tendency in the Monocotyledons (see Table VII). In the account of the first series of these observations (Rea and Small, 1926) the Archichlamydeae were apparently more alkaline than the Sympetalae, but further investigation of a larger number of species has removed this distinction. The proportion of 'acid' to 'alkaline' species for any particular tissue is remarkably similar in the two groups, even in the hairs of the stem. This result shows best when the 'all-acid' families are deducted (see Table VII).

The following families may be considered more or less 'alkaline' as regards these tissues, cortical and medullary, of the flowering stem (so far as this extended series of observations goes) — Chenopodiaceae, Caryophyllaceae, Ranunculaceae, *Papaveraceae*, Leguminosae, *Labiatae*, Scrophulariaceae, Galieae, Valerianaceae, Compositae; also in so far as cortex and *central* pith the Cruciferae Umbelliferae, *Dipsaceae*. All these, except the three in italics were mentioned in the first analysis (Rea and Small) but further work has shown the Compositae to be decidedly more 'alkaline' then 'acid'.

Certain families even on further investigation, show decided 'acidity' extending through all or practically all the tissues of the young flowering stem and, where the leaves have been examined, this acidity shows also in other parts of the plants. These families are Polygonaceae; Crassulaceae, Saxifragaceae, Ribesiaceae and Rosaceae; Geraniaceae and Tropaeolaceae; Cactaceae. It is interesting to note that all these 'acid' families occur in the Archichlamydeae and that no indication of a generally acid family has so far been found amongst the Sympetalae. An unimportant point of some interest is the general alkalinity of *Limnanthes Douglasii*. This genus was removed from the Geraniaceae upon a rather obscure difference in the orientation of the ovule and placed in the Sapindales. The present work shows the Geraniaceae and its allied families to be of an 'acid' type, differing fundamentally in cell reaction from the genus *Limnanthes*.

Certain species in other families also show this same general 'acidity' of all tissues in the flowering stems examined — e. g. *Orchis maculata*, *Chenopodium album*, *Trollius europaeus*, *Brassica campestris*, *Reseda luteola*, *Vicia faba* (flrg. stem only), *Ampelopsis hederacea*, *Euphorbia*, *Hypericum quadrangulum*, *Primula vulgaris*, *Calystegia sepium*; also *Scutellaria galericulata*, *Salvia officinalis*, *Mentha viridis*, *Solanum dulcamara* (flrg. stems only); *Veronica* *chamaedrys*, *Sherardia arvensis*, *Tanacetum vulgare*, *Cnicus lanceolatus*.

The close relationship of some of the 'acid' families indicates some general physiological relationship analogous to the structural similarities. At the same time the acidity of the Polygonaceae has possibly a different origin to that in the Rosales plexus, although that of the acidity of the Geraniales may be of Rosalian origin. The acidity of succulents, both stems and leaves, e.g. Cactaceae, Crassulaceae, *Mesembryanthemum*, *Kleinia*, *Haworthia*, etc., is well known, but the alkalinity of *Salsola Kali* indicates clearly that succulence and acidity have no exclusive causal connection, a conclusion which is confirmed by the absence of succulence from many of the generally 'acid' species, e.g. *Potentilla palustris*, *Geranium robertianum*. There may well be a halophytic succulence as in *Salsola*; and an acidic succulence where the acid is either a by-product of, or a stage in, one or more peculiar variations of metabolism.

In any case this general survey of stem tissues is sufficiently wide to show the great variation from family to family, or genus to genus, or even in some cases from species to species within the same genus. The few vegetative stems included show sufficient difference from the flowering stems of the same plants to indicate that generalisations concerning hydrion concentration in relation to the physiology of plants must be very carefully examined in relation to the actual facts and variations already observed.

Additional data are given in Appendix III.

CHAPTER XI

VARIATIONS IN REACTION — DIURNAL AND SEASONAL

1. Diurnal, non-succulent. 2. Diurnal-succulent.
3. Maturing changes. 4. Summer and Winter changes.
(a) Stems. (b) Gymnosperm Leaves. (c) Angiosperm Leaves.

The tissue reactions (pH values) recorded in the previous chapter show quite clearly that the metabolism of plant species varies. The type of metabolism may be characteristic of the group, family or genus, or it may vary within the same genus or even from part to part of the same plant. We have now to consider such variations in pH as have been described for the same tissue and it is convenient to divide these into (a) diurnal variations in ordinary plants and in succulents; and (b) seasonal variations associated with maturation changes, or with summer-winter changes in plants which are green more or less all the year round.

1. DIURNAL VARIATIONS IN NON-SUCCULENTS

As previously mentioned ROHDE (1917) detected diurnal variations in *Spirogyra*. Using a H-electrode method he found the sap was of pH 6·73 during the night and fell to pH 5·9—5·5 during the day. Methyl red is described as yellow to "schwach rosa" during the night and red to "schwach blaurote" during the day. He also records that the epidermis of carnation and tulip flowers showed a similar acidification in the light.

TRUOG (1919), using clover, alfalfa, soybean and buckwheat, found that the juices of plants cut in the morning were generally more acid than those of plants cut in the afternoon. CLEVENGER (1919) using a careful H-electrode method found that in the leaves and stems of the cow-pea the pH rises during the afternoon, falls during the night and reaches a minimum in the morning.

Haas (1920) on the other hand records a higher pH value for the juice of corn seedlings with a reduction of normal illumination.

Hurd (1923), using *Zea mais*, various strains of Reid Yellow Dent, and an electrometric method on expressed sap found an irregular variation in morning and afternoon saps.

pH values for various strains	Morning .	5·68	5·54	5·60	5·55	5·39	5·52	5·48
	Afternoon .	5·68	5·61	5·58	5·56	5·46	5·55	5·44

"Evidently the diurnal change in acidity, reported in many plants is not marked in corn grown under these conditions." With wheat the same worker (Hurd, 1924) did, however, find clear evidence of a fluctuation in wheat sap pH values for morning (lower) and afternoon (higher), without any corresponding fluctuation in the total acidity.

Total acidity is frequently higher in the morning than later in the day (see Czapek III, p. 102); this has been noted also for algae in the tidal zone (Clark, 1917).

2. DIURNAL VARIATION IN SUCCULENTS

Gustafson (1924) noted variations in pH in *Bryophyllum calycinum*. He found that on a cloudy day there was a pH gradient in this plant, the oldest leaves having the higher pH values and the youngest leaves the lower pH values; while on a sunny day the pH gradient was reversed, the oldest being lowest and the youngest highest in pH values. It is stated that on a cloudy day a decrease in pH was associated with an increase in total acidity; while on a sunny day there was no correlation between pH and total acidity, but see below and figures 14 15. Gustafson (1924) notes the earlier observations by Heyne and others on changing acidity, then writes "As far as the writer is aware, the fluctuation in H-ion concentration has not been reported before." This appears to be true for *Bryophyllum*, although Truog, Clevenger and Haas had noted a similar fluctuation in the pH of the juices of ordinary crop plants (see above). Later (1925), Gustafson reported in more detail. He found that juice expressed from the young parts of *Bryophyllum* showed no change in pH on boiling or on standing for several days, but that the expressed juice varied with the time of day or with the illumination when the juice was collected. Figure 14 shows typical results for a sunny day and figure 15 shows the different results for a cloudy day, presented so that comparison is easier than with Gustafson's figures. The second experiment (fig. 15) was started at 4 a. m. (pH 3·82) and carried on to 10 p. m. The experiments of which

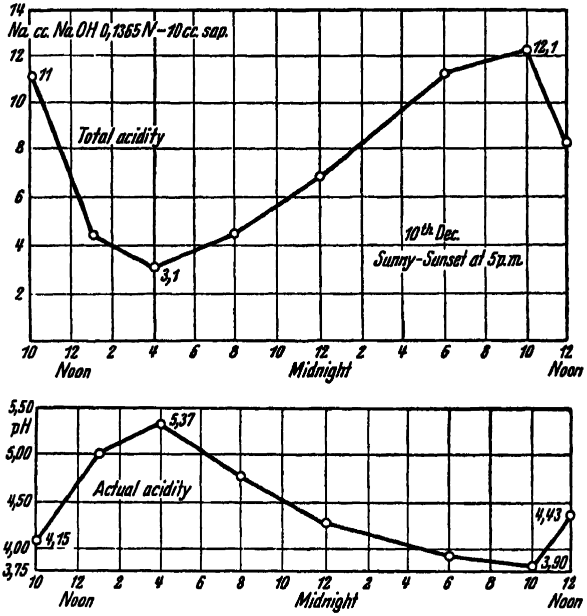

Fig. 14. Diurnal variation in actual and total acidity in *Bryophyllum* (after Gustafson.)

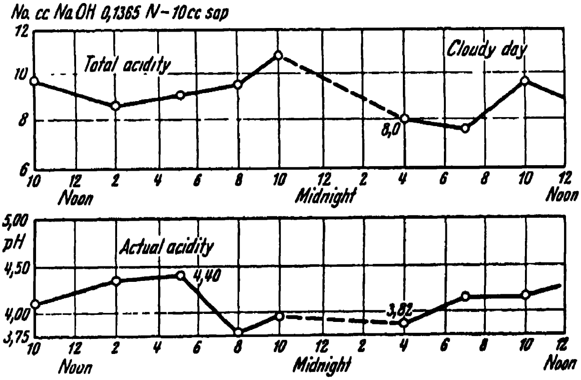

Fig. 15. Diurnal variation in actual and total acidity in *Bryophyllum*, with a cloudy day, showing lack of correlation. (after Gustafson.)

fig. 14 is typical are the only records of the pH of any plant throughout a twenty-four hour period and as such have a special value.

From these curves it will be seen that under sunny conditions an increase in actual acidity (decrease in pH) is associated with an increase in total acidity and *vice versa*; but under cloudy conditions the pH and the total acidity are not correlated in the same way. This from GUSTAFSON (1925) is the reverse of what is stated in his (1924) paper, e. g. "on a clear day, there was no relation between H-ion concentration and total acid."

The phenomena appear to be complex and to involve at least two limiting factors. Bright light reduces both actual and total acidity, while less bright light appears to reduce actual acidity, without affecting the total acidity. The variation in pH is controlled, therefore, by (a) the total concentration of acid and (b) some other factor apparently connected with the metabolism of the living leaf, possibly the type of acid or buffer system produced during medium illumination. In this connection the changes of dilute malic acid in sunlight (SPOEHR 1913) into oxalic, glycollic, formic, acetic and carbonic acids may be of significance, since these acids have different dissociation exponents and would alter the pH without, in the case of oxalic, affecting the amount of titratable acid present. It is, however, improbable that in *Bryophyllum* this simple explanation is available, since GUSTAFSON finds that the acid sap extracted from *Bryophyllum* is unaffected by light during four days exposure under varying conditions, and is unaffected also by oxygen supply.

GUSTAFSON (1925) also gives a number of similar results for shorter periods and for plants placed in the dark for varying periods. In continuous darkness over a period of fifteen days the pH falls to a minimum about pH 3·7 in the first three days, rises to about pH 4·5 on the sixth day and there-after is more or less constant about pH 4·5 (fig. 16).

The actual tissues of the *Bryophyllum* leaf have been examined here by Miss M. J. LYNN using the R. I. M. (unpublished work). The data involve only a few pH values, thus red DER-yellow MR = pH 5·6 *ca*; red MR-green BCG pH 5·2 4·4; pink BAN-green BCG = pH 4·4 *ca*; yellow BCG-blue BPB pH 4·0 *ca*. Three of the four ranges are limited and by taking the range 5.2—4·4 as indicating something about the change point of the indeterminate BAN, i. e. pH 4·8 *ca* we can graph the results and compare them with those found by Gustafson with the *Bryophyllum* in continuous darkness over a period (figure 16). It will be seen that the lower pH values found by Gustafson did not show in Lynn's material, but the previous day was probably cloudy in Belfast. Otherwise the results are in very close agreement, with a shortening of the general period of sway and a considerable variation in the period of sway for each tissue. The acid mucilage cells occur in the hypodermis and bundle sheath and remain pH 4·0 *ca* all the time. Gustafson's data for the juice represent an average of all the tissues in the leaf. Miss Lynn using the R.I.M. found the diurnal variation to be of a similar range, passing to pH 5·6 *ca* in the afternoon for all tissues except xylem, phloem and the mucilage cells.

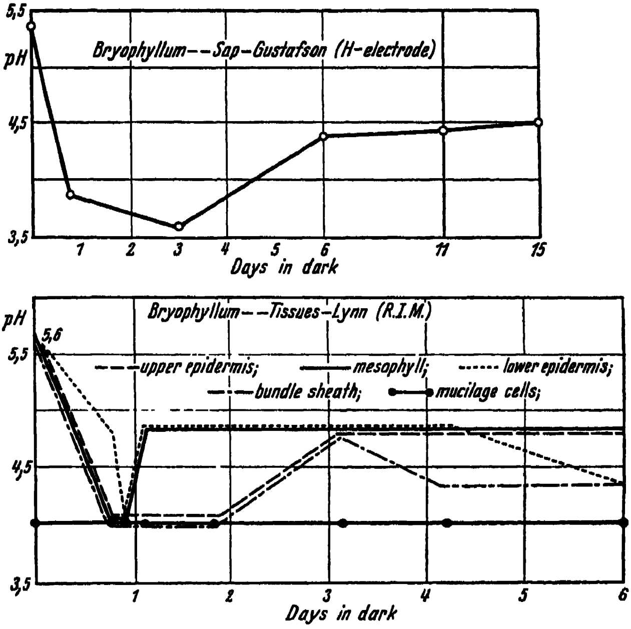

Fig. 16. Comparison of sap data (Gustafson) and tissue data (Lynn), for *Bryophyllum* exposed to continuous darkness.

As Gustafson points out the phenomenon of changes in sourness and total acidity in *Bryophyllum* had been reported upon previously by Heyne (1815), Link (1820), Mayer (1875), KRAUS (1883—86), WARBURG (1886 -88). (see also p. 82 and RICHARDS 1915 for literature.)

Other succulents have also occupied attention from an early period. ASTRUC (1903) is particularly good on the diurnal variations in total acidity of succulents and other plants, but few records of diurnal variations in pH are available. ÚLEHLA (1927) gave some advance data and in (1928) gave details for *Opuntia phaeacantha*. The pressed sap gave the following values - 6·0 a. m. pH 3·5 --3·8; 6·45 a. m. pH 1·4; 8·30 a. m. pH 4·5; 2·30 p. m. pH 6·0; 4·0 p. m. pH 5·5. These sap samples were not all derived from the same plant nor were the pH values determined as a series; the data are incidental to an investigation of the regulation of the external pH by cactus tissue. ÚLEHLA also states that *Rheum undulatum* shows a similar diurnal periodicity in pH; the cell sap from the leaf parenchyma being ac'd in the night and less acid in the afternoon.

HEMPEL (1917), however, gives a large number of data for the expressed juice of succulents which had been exposed to light or darkness for various periods. These may be taken as studies in diurnal variations, and will be found in Chapter XV, which deals with succulents as a special case. The general arrangement is again lower pH values from darkened plants and higher pH values from plants exposed to light.

So far as the records available indicate, we have the action of light with a general tendency towards increasing the pH of plant tissues; this agrees with the observations of WEBER, SCARTH and SAYRE on guard-cell pH values, but the observations by ROHDE on *Spirogyra* and by HAAS on corn seedlings indicate a decrease of pH with greater illumination or an increase of pH with less illumination. GUSTAFSON and others have also noted a lower pH in the morning after a previous sunny day than after a dull day. The available food supply appears to be involved, a suggestion which is supported by the metabolism of succulents and by GUSTAFSON'S records for *Bryophyllum* in continuous darkness. ROHDE'S result may be a consequence of a low metabolic activity in *Spirogyra*, giving an increased acid content only during active photosynthesis and something like the rise in a six days starved *Bryophyllum* during the night in this alga. HAAS'S result (1920 pp. 364 sqq.) with corn seedlings, green and etiolated, is in the same sense. Young (10 days) seedlings show a smaller difference in pH (juice) between green plants and those etiolated by darkness, than do older (13 days) seedlings, while the older (more efficiently metabolising ?) seedlings show a lower pH value than the younger seedlings throughout.

HURD'S (1923) results with vigorous and stunted growths of *Zea mais* may also be significant in this connection. Vigorous plants showed a correlation between pH and total acidity, and the pH of leaf sap (5·42) lower than that of stem sap (5·51); while stunted plants showed no correlation between pH and total acidity, and the pH of leaf sap (5·43) higher than that of stem sap (5·19).

3. MATURING CHANGES IN REACTION

Leaves. The observations by HAAS, GUSTAFSON, MUKERJI and others of a gradient in pH from young leaves (more acid) to older lower leaves (less acid) might be taken as a maturation change involving an increase of pH with age in leaves, but the reversal of the gradient in *Bryophyllum* on a sunny day (GUSTAFSON 1924) may indicate that this change is controlled not so much by age as by enviromental conditions. Age might well be the controlling factor under medium illumination, while light may be the controlling factor when it reaches a certain degree of intensity.

Shoots. HURD (1924), using an electrometric method on several varieties of wheat found that in general the pH value of the sap, *taken at the same time of day*, remained more or less constant for about the first three months and that this period is followed by a decrease in pH associated with the pre-ripening stage, a relatively low pH value being maintained during the flowering and later stages. The values for the different varieties vary considerably, but for the early months of growth the values usually lie between pH 6·3 and pH 5·9, while in the later stages they fall to pH 5·6. Except in two or three of the many records the constant early pH values are not correlated with an observed regular decrease in total acidity from the second to the sixth week, but the later fall in pH is correlated with a later rise in total acidity. This rise in actual and total acidity in wheat is, according to HURD, associated with the rate of drying rather than with head formation or kernel development.

In a later paper the same worker (HURD-KARRER 1928) gives for wheat juice the following data —

Age in Weeks	Stage	pH value	
		Jenkin Wheat	White Odessa Wheat
12	shooting		5·74
13	shooting	5·77	5·81
14	shooting	5·77	
15	shooting		5·74
15	heads in boot	5·74	
15½	heading	5·78	
16	heading	5·74	
16	shooting		5·81
17	heading		5·73
17	flowering	5·77	5·85
18	late flowering		5·77
18	kernels-milky	5·76	
19	kernels-milky		5·73
19	kernels soft dough	5·71	
20	kernels soft dough	5·66	5·83
21	kernels soft dough		5·55

The titratable acid during the maturation period increased in both varieties almost regularly, in cc. N/20 NaOH, from 6·2 to 11·6 for JENKIN and 7·8 to 14·7 falling to 13·1 in the last stage for WHITE Odessa. The maintenance of the pH within narrow limits during this rise in total acidity is attributed, with a considerable degree of probability, to the efficiency of the buffer-systems present. In fact, it appears to the writer that many of the records of pH values (especially between pH 5 and pH 7) for plant juices are to be correlated with metabolic changes in the buffer-systems rather than with increased or decreased production of acids. Given an inactivation of part of the buffer system, by utilisation in the cytoplasm or otherwise, the pH would change without the production of a greater or a lesser quantity of actual acid, the dissociation ratios of which would vary with the concentration of the buffer system (see pp. 66 and 73).

Other maturing changes in particular tissues leading to lignification and acidification in walls or leading to a decrease in acidity in chlorenchyma are mentioned in Chapter X and also below (pp. 148, 195, 196).

Fruits. Using the hydrogen-electrode, which is not subject to the carbon dioxide error after the first determination of this series, BARTHOLOMEW (1923) obtained the following pH values for the lemon at different stages of maturity —

Dates 1920—21	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
Stylar End . .	4·46	2·91	2·64	2·54	2·60	2·57	2·33	2·30	2·23	2·29	2·29
Stem End . . .	4·46	3·08	2·71	2·50	2·64	2·54	2·36	2·33	2·27	2·33	2·33

BARTHOLOMEW notes that the total acidity of the lemon increases with age but the actual acidity varies little after the lemon has reached a diameter of about 3·8 cms. An efficient buffer system is indicated at an early stage (see lemon juice curve, fig. 28). HEMPEL (1917) recorded a similar reaction for lemon juice, pH 2·19—2·25. Lime juice is still more acid, quoted by ATKINS (1922) together with an unnamed Indian berry, as pH 1·7.

These results are amongst the few determinations of pH values for plant juices which may be regarded as something more than residual values (see p. 86 and 132).

GUSTAFSON (1927) made a similar study of the pH of the tomato (JOHN BAER var.) during its ripening. He found that the pH of the juice gradually fell to the fifth week and then rose slightly; while the total acidity fell slightly during the first three weeks and then rose towards the fifth week, falling again with a rise in pH (5th--7th weeks), and rising slightly with a continued rise in pH during the 8th week. There is thus some correlation of pH and total acidity, but during the 1st—3rd and 8th weeks the changes are not correlated. The pH figures are given graphically and are approximately 4·9, 4·9, 4·8, 4·2, 4·3, 4·35 for the 1st, 2nd, 3rd, 5th, 7th and 8th weeks respectively. GUSTAFSON notes that low pH values are associated with the period of rapid growth, and wisely states "whether it is the cause of the rapid growth or the result, is a question which the writer is not ready to answer." The low pH values may be neither cause nor effect but concomitant phenomena, see "pentosan metabolism", Chapter XV.

Seeds and Germination.

DELEANO (1909) found an increase of acidity in the sap of *Ricinus communis* up to a maximum on the eighth day after germination began. MILLER (1910) found an increase in free fatty acid in the hypocotyl of *Helianthus annuus* on germination. ECKERSON (1913) found an increased total acidity in the hypocotyl of several species of *Crataegus* with after-ripening. HEMPEL (1917, p. 116) gives for lupine seedling juice the following electrometric data which show an increase in pH with age —

19 days pH 5·78, 20 days pH 5·93, 22 days pH 6·03; but the total acidity also shows a regular increase thus, in terms of cc. N/5 NaOH to the litmus point, there are —

19 days 3·65, 20 days 6·51, 22 days 7·48.

Rose (1919) found an increased actual acidity ($2{\cdot}00 \times 10^{-7}$ rising to $1{\cdot}18 \times 10^{-6}$ or pH 6·7 to pH 5·93) in *Tilia americana* seeds with after -ripening. Jones (1920) finds an average of pH 8·335 for dormant seeds and an average of pH 7·909 for after ripened seeds. Rose's method was to grind 20 seeds with 25 cc. water and add 100 cc. to the liquid produced; while Jones ground two embryos with 1 cc. distilled water and then added 5 cc. distilled water. Jones noted that the fluid became more alkaline on standing. Both workers used H-electrode method on the fluids obtained. Scarth (1924) determined the pH of freshly distilled water, using a potentiometer and found values around pH 5·5; while pH 7·1 was obtained by boiling off the carbon dioxide in a silica vessel. In view of this fact, the results obtained by Rose, Jones and others — using the method of grinding and extracting with distilled water — are to be regarded not as determinations of actual acidity of natural juices, but as data concerning residual reactions after the sap has been mixed with a solution of carbon dioxide in water (pH 5·5 ca.) and treated in a hydrogen electrode with a stream of gas which removes all the carbon dioxide both from water and from sap and reduces the substances of the aqueous extract until an equilibrium is reached. These data are, therefore, aleatory and very difficult to explain unless all the circumstances are detailed.

Haas (1920) found an increase in total acidity with age (10—13 days) in corn seedlings as did Hempel for the lupine, but he also found a decrease in pH (about 5·8 to 5·3) instead of a rise (5·78 to 6·03) as Hempel found in the lupine. As both sets of data are electrometric the differences are probably not significant in relation to the living tissues on account of the errors of the methods.

Pack (1921) using "Clark and Lubs indicators" found pH values for various parts of Juniper seeds, stored for various periods thus —

pH of seeds during after-ripening, stored at 5° C.

	Dry	30 days	60 days	90 days
endosperm	4·6—6·0	4·6—5·2	4·4—6·0	4·4—5·2
embryo	8·4—8·8	6·8—7·6	6·8—7·6	—
hypocotyl				6·0—6·8
outer cells of embryo				—
and of hypocotyl				4·4—5·2
inner cells of embryo				4·6—6·0

Ives (1923) using "La Motte standard indicators" found that both freshly gathered and dry stored seeds of *Ilex opaca* show a pH value of 6.

Apart from the R.I.M. observations on the sunflower and broad bean (Chapters XII—XIII) which show acidification of some tissues, we have only Pack's data which show a similar decrease in pH. His note on the regular increase of total acidity with after-ripening may be added to the data given by Hempel and Haas on a similar increase with age of seedling plants.

4. SUMMER AND WINTER VARIATIONS

(a) *Stems.*

Hooker (1920) investigating the juice of bearing spurs of apples, found for a variety called Wealthy, a steady pH value with varying total acidity e. g. expressing the latter as cc. N/10 acid per gm. dry weight he found Feby. 2·04, June 3·40, Sept. 1·74 while the corresponding pH values were 5·8, 5·7 and 5·9.

Abbott (1923) on the other hand found marked seasonal changes in the pH of both apple and peach, in general a high pH in summer, and a drop in the autumn, followed by a rise in November, which is maintained through the following spring. Gillespie's colorimetric method was used throughout. Abbott suggests that the low pH in September may be correlated with the high sulphur content, but he makes a further suggestion in relation to phosphate content and buffering which is discussed later (Chap. XIX). As these results appear to be the only data available for comparison with the R. I. M. results they are given as a graph (figure 17).

The tissue reactions of a number of selected herbaceous and woody stems, examined at monthly intervals throughout the year, were reported upon by Rea and Small (1927) as follows.—

STEM TISSUE REACTIONS THROUGHOUT THE YEAR

METHOD

The Range Indicator Method as previously described was used throughout. Transverse sections of the vegetative stems were examined. Five indicators were used up to and including December 1925, and six indicators were used during 1926. This work was started in 1924, but a break of several months occurred in the first series of results, and it was thought better to leave these unpublished and present an unbroken sequence of observations for one period of twelve months, June 1925 to May 1926.

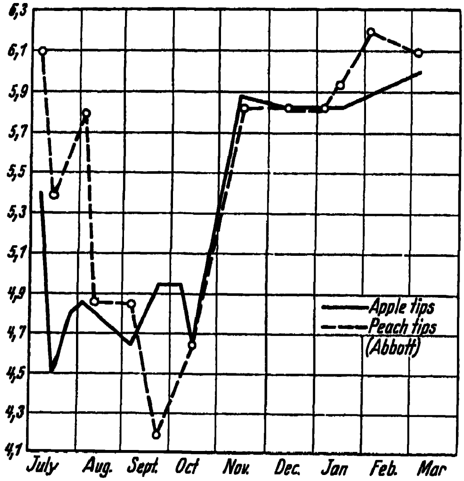

Fig. 17. Seasonal variation in the pH of expressed juice of apple and peach tips. (after Abbott.)

At least three stems from each kind of plant were taken each month, and two or three sections of each part, upper, middle and lower, of each stem were examined, after immersion in the indicator solutions. In general nine tissues were differentiated and epidermal hairs or cuticle usually added a tenth observation to the series.

We recognise very clearly that we are only at the beginning of this work, and desire to point out that the results must be regarded as a general survey of the possibilities for more intensive work.

The method of analysis and presentation of the pH ranges as letter symbols may appear to be unnecessary complications, but we would ask other workers in this field to consider the number of observations necessary for the production of the data given in the Tables which are here included.

Two or 3 sections each of 3 stems were taken, giving 6—9 sections; 5 indicators were used during the first 7 months giving 210—315 sections, and 6 indicators were used during the last

5 months giving 180—270 sections or a total of 390—585 sections per year. In general 10 parts or tissues were observed in each section giving 3900—5850 observations; nine different species, one being duplicated for light and shade conditions, were examined giving 39,000—58,500 observations. Then in all cases each stem was divided into upper, middle and lower parts, so that the total of the observations required for the data here presented amounts to something between 117,000 and 175,500, or a mean of approximately 150,000.

All these observations were originally recorded as the colour given by each tissue in each section in each indicator. By arranging the records in the order of the ranges or indicators as given (p. 46) and marking each record C for acid and K for alkaline indications, the change point in the series, and hence the range, was determined. The ranges as found in this way were card-indexed, the data for the upper, middle and lower parts of each stem being on one card. The data were then entered as letter symbols in our pH ledger. In any case where two or more of the upper, middle and lower parts gave the same data for all tissues a further condensation became possible as shown in the Tables now published. Sometimes also it was found that the data for two or all three of the stems were the same throughout, and then more condensation became possible.

This process of repeated 'boiling down' has been applied in the production of all the Tables in this series, and it is necessary in order to obtain the data in a condition for publication. It is necessary also as an escape from the confusion of numerous numerals, in order to see clearly the changes and differences which occur.

RESULTS

The stems examined may be divided into two groups, 1. more or less herbaceous, and 2. woody. The data for the different species are discussed briefly below. The difficulty of presenting a series of thirty-six lines of letter symbols has been met by combining the results for the upper U, middle M and lower L parts of the stem wherever possible. In some cases square brackets [ ] have been used to mark the second of a pair of parts where U and M or M and L differed by only one or two letters. Ordinary brackets indicate abnormal reactions as before.

The records are condensed by means of the system of a letter for each range as previously explained. The indicators, when five were used, did not include bromo-cresol green; where six indicators are given the full normal series as described was in use.

In the case of the woody stems three twigs were taken from each shrub, from the top, side and base of the shrub. These are indicated in the Tables as T. S. and B. respectively.

HERBACEOUS STEMS

Cerastium tomentosum

The vegetative stems of this plant were examined in transverse section. The material was obtained from the Class Garden, Queen's University, where the plants were growing in an exposed position with a southern aspect.

It is of some interest to compare the present results for this species (Table I) with those given in our first series. The **b** range pH 5·9 to 5·6 includes the **c** range, approximately pH 5·6. In the same way the **Z** range pH 5·2—4·0 includes **e** or pH 5·2—4·8, **f** or pH 5·2—4·4, **g** approximately pH 4·4, **h** or pH 4·4—4·0 and **i** approximately pH 4·0. It should be noted that the former results were obtained from the flowering stems as in this case.

As the herbaceous plants were chosen with a view to determining their tissue reactions throughout the year, the choice was limited to a few species which in most cases develop the flowering stem as an elongation of the ordinary vegetative stem. The tissue reactions might, therefore, be expected to fall within the same ranges as before. In the case of *Cerastium tomentosum*, although the present ranges do not always coincide and are not always included in the older data, the results now given are in general consistent with those previously given and now repeated at the foot of Table I.

The *xylem* is the tissue with the most constant reaction throughout the year. The **h** range pH 4·4—4·0 includes the **g** range approximately pH 4·4. Whenever bromo-cresol green (B.C.G.) was added to the series, it became possible to be more precise and the pH 4·4—4·0 of June to December 1925 became 4·4 of January to May 1926. This phenomenon was more or less usual in the other species examined.

Table I. *Cerastium tomentosum*

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems Size in cms
1925												
June . . .	U	e	a	a	a	a	a	h	a	a	e	
,, . . .	M	a	a	a	a	a(h)1)	a	h	a	—	e	
,, . . .	5 L	a	a	a	a	hk	c	h	a	—	e	1 6·5
,, . . .	U	e	a	a	a	a	a	h	a	a	e	4·0
,, . . .	5 ML	a[e]	a[c]	a	a	ah1)	a[c]	h	a	—	e	2 5·0
July . . .	U	a	a	a	a	a	c	h	c	c	e	
,, . . .	5 ML	a[e]	a[e]	a	a	eh1)	e	h	e	—	e	3 5·0
August .	UM	a	a	a	a	a	a	h	a	—	d	
,, . .	5 L	a	a	a	a	hk1)	a	h	a	—	d	3 7·5
September	U	a	a	a	a	a	a	h	a	a	d	
,,	5 ML	a	a	a	a	ah1)	a	h	a	—	d	3 7·5
October. .	U	e	a	a	a	a	a	h	a	a	e	
,, . .	5 ML	e	a	a	a[e]	ah1)	a[e]	h	a	a	e	1 6·5
,, . .	U	e	a	a	a	a	a	h	a	a	e	2 6·5
,, . .	5 ML	e	a	a	a	ah1)	a	h	a	a	e	4·0
November.	U	c	c	a	e	e	c	h	c	c	e	5·0
,, .	5 M	c	e	a	c	eh1)	e	h	c	e	e	3 5·0
,, .	L	a	a	a(h)1)	c	eh1)	c	h	e	c	e	
December.	U	a	a	a	a	a	a	h	a	a	e	
,, .	5 M	e	a	a	a	eh	a	h	a	a	e	
,, .	L	e	a	a(h)1)	a(h)1)	hk	e	h	a	a	e	3 7·5
1926												
January .	U	a	a	a	a	a	a	g	a	a	e	
,, .	6 ML	e	a	a	e	g	e	g	a	a	e	3 6·5
February .	UM	e	e	e	e	e[g]	e	g	e	e	e	
,, .	6 L	e	e	e	e	eg1)	e	g	e	e	e	3 6·5
March . .	UM	e	e	e[a]	e	e	e	g	e	e	e	
,, . .	6 L	a	a	a	e	eg1)	e	g	e	e	e	3 6·5
April . . .	U	e	e	a	a	a	e	g	e	e	e	6·5
,, . . .	6 M	e	e	ag1)	e	ah1)	e	g	e	e	e	6·5
,, . . .	L	a	a	ag1)	eg1)	agk1)	c	g	e	e	e	3 5·0
May . . .	U	a	a	a	a	a	a	g	a	a	e	
,, . . .	6 M	c	c	a	a	ag1)	a	g	a	a	c	3 6·5
,, . . .	L	c	c	a	a	hk1)	a	g	a	a	c	
First series		b	b	b	b	bZ	b	Z	b	b		

1) = reaction of walls.

The range d pH 5·6—4·8 includes e pH 5·2—4·8, and if this be kept in mind the reaction of the *epidermal hairs* is seen to be very constantly in the region of pH 5·6—4·8. The only month in which variation to pH 5·6 occurred was October. A similar variation is possible for August and September, so that after the flowering period the hairs upon the leaves may have a slightly different reaction. This may, of course, be due rather to buffer changes consequent upon a better supply of inorganic salts rather than to autumnal metabolic changes.

The reaction of the *epidermis* varies from pH 5·9 to pH 5·6 and this variation is exceeded only in January where the middle and lower parts of the stem were more acid, pH 5·2—4·8. What might be described as the 'winter' reaction, pH 5·6, for the epidermis extends with exceptions in various parts of the stem from October to May. Two out of the three parts have a reaction more acid than pH 5·9 during these eight months. In June two of the three parts are at pH 5·9 in most stems, while that reaction is very constant for July, August and September.

The *sub-epidermis* is more constant in reaction. With the exception of one stem [c] in July, the pH is 5·9 or 5·6. The more acid range pH 5·6 appears in November and occurs at intervals until May, but from June until October the reaction is practically constant around pH 5·9.

Apart from occasional records of apparently lignified cell walls, the *cortex* shows the same variation as the epidermis and sub-epidermis, with a further shortening of the 'winter' condition to February and (in part) March.

This 'winter' condition extends from October to May for the epidermis, from November to May for the sub-epidermis, and from February to March for the cortex.

The *endodermis* shows a change from pH 5·9 to pH 5·6 in November, but reverts to the former reaction in December. The winter climate in Belfast is remarkably mild, and this might be a weather effect. This suggestion receives support from the increased acidity during the following months January to April, when the weather is apt to be more severe, together with a return to pH 5·9 in May when the summer conditions begin to be more or less favourable.

The reactions for the *pericycle* indicate lignification of the cell walls in this tissue, especially in the middle and lower parts of the stem. The reaction of the cell contents varies somewhat erratically, from pH 5·9 to pH 5·6 and in a few odd months (July, November and December), was more acid pH 5·2—4·8 in the middle and lower parts of the stem. A variation in reaction during the process of lignification might be possible, although as yet we have no conclusive evidence that the cell contents are acid while lignification is in active progress.

The *phloem* shows a variation in reaction within the range pH 5·9 to 4·8. During the period February to April when the plant is merely vegetating the reaction is constant around pH 5·6. In May and June when assimilation is most active and still free from floral complications the reaction is more or less constant at pH 5·9, with a lag at pH 5·6 in the lower part of the stem sometimes. In July, the main flowering period, the acidity increases to pH 5·6 or pH 5·2—4·8, followed by a return to vegetative activity and pH 5·9 in the period August to October. During the winter, November to January, the reaction varies erratically, possibly with local weather conditions.

The constant reaction of the lignified cell walls of the xylem has already been pointed out. The xylem parenchyma was not recorded separately from the medullary ray region of the pith.

The reaction of the *pith* both in the region of the vascular bundles and in the central region varied from pH 5·9 to pH 5·6 in such an erratic fashion that no metabolic or climatic factors can be suggested at present. This is, of course, in accordance with the lack of any very definite metabolic function for the pith and with its central, more or less protected position.

Cheiranthus cheiri

The material of this species was obtained from two different gardens. Plants from an exposed position with a southern aspect were obtained at Queen's University and gave the data in Table II. Plants were also obtained elsewhere from a shaded position with a northern aspect and gave the data in Table III. The results are not strictly comparable because, while the first group varied in age from two to thirteen months, the second or shade plants were all over one year old.

Transverse sections of vegetative stems were studied. If we compare the resulting data with those given previously for the flowering stem, there is an agreement of the Z range with g

Table II. *Cheiranthus cheiri* (*Sun*)

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems	Size of stems	Age in months
1925														
June . . .	5 UML	a	a	a	a	a	a	h	a	a	a	3	4·0	12
July . . .	UM	a	a	a	a	a	a	h	a	a	a			
„ . . .	5 L	a	a	a(e)′	a	a	a	h	h	a	a	3	6·5	13
August . .	5 UML	a	a	a	a	a	a	h	a	a	a	3	4·0	2
September	UM	a	a	a	a	a	a	h	a	a	a			
„	5 L	a	a	a	a	a	a	h	ch	a	a	3	10·0	3
October. .	UM	ac	a	a	a	a	a	h	c	a	a			
„ . .	5 L	ac	a	a	ac	a	a	h	c(c)	a	a	2	7·5	4
„ . .	5 UML	c	c	c	c	a	a	h	a	a	a	1	7·5	4
November.	5 UML	a	a	a	a	a	a	h	a	a	a	2	6·5	5
„ .	UM	a	a	a	a	a	a	h	a	a	a			
„ .	5 L	a	a	a(e)′	e	a	a	h	a	a	a	1	6·5	5
December.	5 UML	a	a	a	c	a	h	h	h	h	a	3	6·0	6
1926														
January .	6 UML	ae	a	a	a(e)	a	a	g	a	a	a	3	7·5	7
February .	6 UML	a	a	a	a	a	a	g	a	a	a	3	7·5	8
March . .	6 UML	a	a	a	a	a	a	g(h)	a	a	a	3	8·5	9
April . . .	6 UML	a	a	a	a	a	a	g(h)	a	a	a	3	8·5	10
May . . .	UM	a	a	a	a	a	a	g	a	a	a			
„ . . .	6 L	a	a	a	a	a	a	hk	a	a	a	3	6·5	11
First series		b	b	b	—	—	b	Z	bZ	Z				

(e) groups of cells in endodermis. (e)′ collenchyma. (h) in one stem.

or h and a generally less acid a range instead of the former b range, but the difference between approximately pH 5·9 and pH 5·9—5·6 is very slight. Further work may show that it does in fact exist as a difference between the flowering and the vegetative stem, and the wallflower would then be another example of those plants with flowering stems more acid than the vegetative stems, already mentioned (Chapter X).

Apart from one slightly more acid (pH 5·6) stem in October (Table II), the uniformity of reaction in the tissues of *Cheiranthus cheiri* is remarkable. This, as will be seen later, is not an isolated

Table III. *Cheiranthus cheiri* (*Shade*)

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems	Size in cms	Age
1925														
June . . .	UM	a	a	a	a	a	a	h	a	a	d			Over
,, . . .	5 L	c	a	a	a	a	a	hk	a(h)	a	d	3	7·5	one year
July . . .	UM	a	a	a	a	a	a	h	a	a	d			
,, . . .	5 L	c	a	a	a	a	a	h	h	a	d	3	5·0	,,
August . .	5 UM	a	a	a	a	a	a	h	a	a	d			
,, . .	L	a(h)′	a	a	a	a	a	h	h	a	d	3	7·5	,,
September	UM	a	a	a	a	a	a	h	a	a	d			
,,	5 L	a	a	a	a	a	a	h	h	a	d	2	7·5	,,
,,	5 UML	a	a	a	a	a	a	h	a	a	d	1	7·5	,,
October. .	UM	a c′	a	a	a	a	a	h	a	a	d			
,, . .	5 L	a	a	a	a	a	a	h	a c′	a	d	3	8·5	,,
November.	UM	a	a	a	a	a	a	h	a	a	a			
,, .	5 L	a	a	a	c	a	a	h	a	a	a	3	7·5	,,
December.	UM	a	a	a	a	a	a	h	a	a	a			
,, .	5 L	a	a	a	a(c)′	a	a	h	a h	a	a	3	10·0	,,
1926														
January .	UM	a	a	a	a	a	a	g	a	a	—			
,, .	6 L	a	a	a	a(c)′	a	a	g	a	a	—	3	7·5	,,
February .	UM	a	a	a	a	a	a	g	a	a	—			
,, .	6 L	a	a	a	a(c)	a	a	h	a	a		3	7·5	,,
March . .	UM	a	a	a	a	a	a	g	a	a				
,, . .	6 L	a	a	a	a	a	a	h	a	a		3	7·5	,,
April . . .	UM	a	a	a	a	a	a	g	a	a	—			
,, . . .	6 L	a	a	a	a	a	a	h	a	a	—	3	7·5	,,
May . . .	UM	a	a	a	a	a	a	g	a	a	—			
,, . . .	6 L	a	a	a	a	a	a	hk	a	a	—	3	7·5	
First series		b	b	b	—	—	b	Z	bZ	Z				

(c)′ in one stem. (c) collenchyma. (h) groups of cells. (h)′ cuticle. c′ walls.

phenomenon. The fact that about thirty thousand observations can be made upon seventy two plants of one species, thirty six from a sunny position in one garden and thirty six from a shaded position in another garden, without yielding more than a few variations in the hydrogen ion concentration of the tissues bears witness, not only to the stability of the reaction in this species throughout the year and under different conditions, but also to the reliability of the Range Indicator Method. We do find variation and differentiation in a considerable number of other cases, so that if variations or differentiations of any size occurred they would be and are detected by this method.

Considering the tissues, the *pericycle* is uniformly of pH 5·9 with no exception in either sun or shade plants and throughout the year. The *sub-epidermis*, *cortex*, *phloem* and the central part of the *pith* were all of pH 5·9 throughout the year in the shade plants (Table III). In the sun plants there is one exception, pH 5·6, in the sub-epidermis of one plant (Oct.); in the phloem and central pith there is also one exception, pH 4·4—4·0 in December, while in the cortex of the sun plants an occasional development of collenchyma gave pH 5·2—4·8 in addition to the pH 5·6 for one plant in October as for the sub-epidermis.

The *epidermal hairs* were of pH 5·9 throughout the year in the sun plants, but of pH 5·6—4·8 during the summer and autumn in the shade plants, becoming less acid, pH 5·9, in the winter. The *epidermis* was mainly of pH 5·9 and, apart from the October sun plant (pH 5·6), greater acidity was found only occasionally in the walls or in the contents of some of the cells. In the shade plants for June and July the epidermis of the lower parts showed pH 5·6 as compared with pH 5·9 for upper and middle parts of the stems.

The *endodermis* was at pH 5·9 uniformly during the months March to September in both sun and shade plants. Slightly greater acidity appeared in the sun plants for October, and still greater acidity appeared in both sun and shade plants during November, December and January. The sun plants showed this increase of acidity earlier (Oct.) and lost it earlier (Feby.), than did the shade plan's which showed the increase of acidity in November and returned to the pH 5·9 in March.

The *xylem* again showed a change from h to g with the introduction of the sixth indicator, but the bromo-cresol green indications were not always certain, so that the h range, pH 4·4—4·0, occurs also in the later records. In a few cases there were still more acid parts in the xylem with pH $<$ 3·4. The *pith* in the region of the medullary rays was mainly of pH 5·9, but the lower parts of the stem during 1925 showed a general tendency towards lignification with concomitant increase in acidity. During 1926 this ray region of the pith is recorded as uniformly of pH 5·9 but this requires re-investigation in view of the above-mentioned records for 1925.

From these brief notes on the records in Tables II and III, it will be seen that, in addition to the already noted uniformity, there are also variations which appear to have some significance in the general life of the plants. There are slight differences in sun and shade plants and there are slight differences which are apparently connected with seasonal changes rather than with age or state of development of the plants.

Lamium purpureum

The material of this species was obtained from plants cultivated in an exposed position with a southern aspect in the Class Garden, Queen's University. The vegetative stems which grow up into flowering stems were examined in transverse section. The results are summarised in Table IV.

On comparing these records with those for the flowering stem, we find that the records for all the tissues are consistent, with the exception of **bZ** for the endodermis. The more acid range is not found in the endodermis of the vegetative stems, and the **Z** range, pH 5,2—4,0, is confined in that tissue to the lower part of the flowering stem. The upper and middle parts have the **b** range which is very near the **a** and includes the **c** range found for the endodermis of the vegetative stem.

Considering the present data for the tissues, the *collenchyma* and the *epidermal hairs* are uniformly of pH 5·2—4·8 throughout the year. The *xylem* shows the range pH 4·4—4·0 restricted to pH 4·4 by the consistently definite indications given by bromocresol green. The *epidermis* being self-coloured with a decided pink yielded no reliable indications of reaction values. The reaction of the *sub-epidermis* varied, being mainly pH 5·6 but showing greater acidity in June and slightly less acidity in July, and from December to March in some or all of the plants examined. June is the main flowering period and the June to July variations may be connected with anthesis, while the other period is that of reduced vegetative activity, the winter period.

Table IV. *Lamium purpureum*

Date	Indicators Part	Epidermis	Sub-Epidermis	Collenchyma	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems	Size in cms.
1925														
June . . .	5 UML	Natural pink colour	e	e	c	c	—	c	h	a	a	e	3	7·5
July	U		a	e	a	a	—	a	h	a	a	e		
,, ...	5 M		a	e	a	a	—	a	h	a	b	e	3	7·5
,, ...	L		a	e	a	a	—	a	h	ah′	b	e		
August. . .	5 U		c	e	a	a	—	a	h	a	a	e		
,, . . .	M		c	e	a	a	—	a	h	a	a	e	3	7·5
,, . . .	L		c	e	a	a	—	a	h	a h′	a	e		
September .	U		c	e	c	c	—	c	h	c	a	e		
,, .	5 M		c	e	c	c	—	c	h	a	a	e	3	10·0
,, .	L		c	e	c	c	—	c	h	c h′	a	e		
October . .	U		c	e	c	c	—	c	h	a	a	e		
,, . .	5 M		c	e	a	a	—	a	h	a	a	e	3	7·5
,, . .	L		c	e	a	a	—	a	h	a h′	a	e		
November .	5 UML		c	e	c	c	—	c	h	c	c	e	3	7·5
December .	5 UML		a	e	a	a	—	a	h	a	a	e	3	6·5
1926														
January . .	6 UML		c	e	c	c	c	c	g	a	a	e	2	5·0
,, . .	6 UM		a	e	a	a	a	a	g	a	a	e		
,, . .	L		a	e	a	c	a	a	g	a	a	e	1	6·5
February. .	6 UML		a	e	a	a	a	a	g	a	a	e	3	6·5
March . . .	U		c	e	c	c	—	c	g	c	c	e		4·0
,, . . .	6 M		a	e	a	a	—	a	g	a	a	e	3	5·0
,, . . .	L		a	e	a	a(c)	—	a	g	a	a	e		5·0
April . . .	6 UML		c	e	c	c	—	c	g	c	c	e	2	7·5
,, . . .	U		c	e	a	a	—	c	g	a	a	e		
,, . . .	ML		c	e	c	c	—	c	g	c	c	e	1	5·0
May	6 UML		c	e	a	a	—	a	g	a	a	e	3	6·5
First series.		—	b	Z	b	bZ	b	b	Z	b	b			

(c) 1 stem only. h′ walls.

The range in the *cortex*, *endodermis* and *phloem* is from a to c, pH 5·9 to pH 5·6. These three tissues vary in the same way, erratically from month to month throughout the year. Local weather or fluctuating internal conditions rather than seasonal changes either inside or outside the plant seem to be indicated in this case as the factors governing the reaction values.

The *pith* between the vascular bundles shows a general tendency towards lignification of the walls as the stems get older, giving pH 4·4 to 4·0 from July to October. The shoots examined from November onwards were new growths, formed after the flowering stems had died down for the winter. The cell contents showed an erratic variation from pH 5·9, which was normal, to pH 5·6 which occurred in some sections in November. The cell contents of the *central pith* showed a similar variation within the same range.

Senecio vulgaris

The material of this species was obtained from plants grown in a sheltered position with a southern aspect in the Research Garden, Queen's University. The records are summarised in Table V, and for all tissues they are consistent with the previous records for the flowering stem which is again an elongation of the vegetative stem.

With one doubtful exception the *collenchyma* is always of pH 5·2 to 4·8. The *xylem* is again of pH 4·4—4·0, restricted to pH 4·4 approximately by the use of bromo-cresol green.

The *epidermal hairs* are of pH 5·6, except in January and February when they are recorded as more acid pH 5·2—4·8.

The *epidermis* and *sub-epidermis* are similar, being pH 5·6 from March until November, with the middle and lower parts of the stem epidermis in May more acid, pH 5·2—4·8. This variation is again in the main flowering season. In all the plants for December and in two of the three in February a lesser acidity of these two tissues, pH 5·9, is recorded.

The *cortex* varies in much the same way as in *Lamium purpureum* from a to c erratically, with the c range predominating. The *endodermis* is more uniformly of pH 5·6, with pH 5·9 appearing in some cells of the lower part of the July stems and in the whole of this tissue in all stems for December, April and May. The *pericycle* and *phloem* vary almost in the same way as the endodermis, except that the pH 5·9 does not occur in April.

The *pith* between the vascular bundles is mainly of pH 5·9, but shows pH 5·6 in September, November, and January to March.

Table V. *Senecio vulgaris*

Date	Indicators Part	Epidermis	Sub-Epidermis	Collenchyma	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems	Size in cms.
1925														
June . . .	5 UML	c	c	e	c	c	c	c	h	a	a	—	3	6·5
July	5 UM	c	c	e	a	c	c	c	h	a	a	c		
„	L	c	c	e	a	c a	c a	c	h	a	a	c	3	6·5
August . . .	5 UMD	c	c	c e	a	c	c	c	h	a	a	c	3	7·5
September .	U	c	c	e	c	c	c	c	h	c	a	c		
„ .	5 M	c	c	e	c	c	c	c	h	c	c	c	2	10·0
„ .	L	c	c	e	c	c	c	c	h	c	a	c		
„ .	5 UML	c	c	e	c	c	c	c	h	c	c	c	1	10·0
October . .	UM	c	c	e	c	c	c	c	h	a	a	c		
„ . .	5 L	c	c	e	a	c	c	c	h	a	a	c	2	5·0
„ . .	U	c	c	e	c	c	c	c	h	a	a	c		
„ . .	5 ML	c	c	e	a	c	c	c	h	a	a	c	1	7·5 10·0
November .	5 UML	c	c	e	c	c	c	c	h	c	c	--	3	7·5 7·5
December .	5 UML	a	a	e	a	a	a	a	h	a	a	c	3	5·0
1926														
January . .	6 UML	c	c	e	c	c	c	c	g	c	c	c	3	5·0
February .	6 UML	a	a	e	a	c	c	c	g	c	a	c	2	5·5
„ . .	6 UML	c	c	e	c	c	c	c	g	c	a	c	1	5·5
March . . .	6 UML	c	c	e	c	c	c	c	g	c	c	c	3	5·0
April . . .	6 UML	c	c	e	a	a	c	c	g	a	a	c	3	7·5
May	U	c	a	e	a	a	a	a	g	a	a	c		
„	6 ML	e	c	e	a	a	a	c	g	a	a	c	3	7·5
First series .		b	b	—	b	b	—	b	Z	b	b			

The *central pith* varies rather more but along the same lines as the outer pith.

WOODY STEMS

Aucuba japonica

The material of this species was obtained from a shrubbery in the grounds of Queen's University. The records are summarised in Table VI.

Table VI. *Aucuba japonica*

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Cuticle	No. of stems	Size in cms.	Part of shrub
1925														
June . .	5 UML	e	e	e	e	e	e	h	e	e	—	1—	5·0	T.
,, . .	5 UML	e	e	e	e	e	e	h	e	e	—	2—	5·0 6·5	S.B.
July . . .	5 UML	e	a	a	a	a	a	h	a	a	—	3—	7·5	T.S.B.
August . .	UM	e	a	a	a	a	a	h	a	a	—			
,, . .	5 L	e	a	a	a	a	a	h (k)	a	a	—	3 –	7·5 6·5	T.S.B.
September	U	e	a	a	a	a	a	h	a	a	—			
,,	5 ML	e	a	a	a	a	a	h (k)	a	a	- -	3 –	10·0 5·0	T.S.B.
October .	U	e	e	e	e	e	e	h	e	e	—			
,, .	5 ML	e	e	e	e	e	e	hk	e	e	—	3—	5·0 8·5	T.S.B.
November	U	e	e	a	a	a	a	h	a	a	—			
,,	5 ML	e	e	a	a	a	a	hk	a	a	—	2 –	4·0	T.B.
,,	U	e	a	a	a	a	a	h	a	a	- -			
,,	5 ML	e	a	a	a	a	a	h	a	a	—	1	6·5	S.
December	5 UML	e	e	e	e	e	e	h	e	e	---	3	6·5	T.S.B.
1926														
January .	6 UM	a	a	a	a	a	a	g	a	a	k			
,, .	L	a	a	a	a	a	a	h	a	a	k	3	7·5	T.S.B.
February .	6 UML	e	a	a	a	a	a	i(k)	a	a	k	3 -	7·5	T.S.B.
March . .	U	eg′	a	a	a	a	a	g	a	a	k			
,, . .	6 ML	eg′	a	a	a	a	a	h	a	a	k	3	7·5	T.S.B.
April . .	U	ae	ae	a	a	a	a	g	a	a	k			
,, . .	6 M	ae	a	a	a	a	a	g	a	a	k	2	7·5	T.S.
,, . .	L	ae	ae	ae	a	a	a	h	a	a	k			
,, . .	6 U	e	e	ae	e	e	e	g	e	e	k	1 --	5·0	B.
,, . .	ML	e	ee	ae	e	e	e	h	e	e	k			
May . . .	6 U	e	e	e	e	a	e	h	e	e				
,, . . .	M	e	e	e	e	a	e	g	e	e	-	1	5·0	B.
,, . . .	L	e	e	e	e	e	e	g	e	e	—			
,, . . .	U	e	e	ae	a	a	a	g	a	a	—			
,, . . .	6 M	e	e	e	e	e	e	g	a	e	g	1—	6·5	T.
,, . . .	L	e	e	e	e	e	e	i	a	e	—			
,, . . .	6 U	e	e	e	e	e	a	g	a	e	—			
,, . . .	M	e	e	e	e	e	a	g	a	e	g	1 – –	5·0	S.
, . . .	L	e	e	e	e	e	e	g	a	e				

g′ walls.

Apart from the practically constant records of strong acidity in the lignified walls of the *xylem* and in the *cuticle*, the tissues of this stem show quite a considerable variation throughout the year. For example, most of the tissues of the top twig in June were of pH 5·6, while the side and base twigs showed pH 5·2—4·8, and all three twigs for July were mainly of pH 5·9.

The *epidermis* shows a tendency to be of pH 5·2—4·8 during the summer months and of pH 5·6 or higher from August to February, with December as an exception. The return to the more acid range was complete in March, but two twigs reverted to the less acid range in April and the upper parts of two twigs were of this higher pH in May. One stem in June also showed the lesser acidity, so that there were several exceptions to the general tendency towards greater acidity during the summer (April in part to July), and lesser acidity during the rest of the year.

The *sub-epidermis* is mainly in the range pH 5·9—5·6 with exceptions in May, June and December. The *cortex* and *endodermis* varied in the same sense as the sub-epidermis.

The *pericycle* showed the range pH 5·9—5·6 with exceptions in April (one twig), in June (two twigs) and in December (three twigs). The *phloem* and *pith* were the same as the pericycle with one additional more acid twig in May.

No clear seasonal variations were found in the twigs of the spotted laurel.

Ligustrum vulgare

This material was from a hedge in the grounds of Queen's University. The results are summarised in Table VII, and are consistent with those recorded in the First Series so far as the latter go.

There is a general tendency for the walls of the cells to be more acid in the lower parts of the twigs, in the *epidermis*, *sub-epidermis*, *pericycle* and *xylem*. The tendency is very clearly shown in the pericycle, where the more acid parts often include both middle and lower parts of the stem, and also by the xylem.

The variation in *epidermis* and *sub-epidermis* is very large extending from the range a to i, that is throughout the normal range of our observations. It will be noted, however, that for the epidermis the higher range a to c does not occur during the

Table VII. *Ligustrum vulgare*

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems	Size in cms.	Part of shrub
1925														
June . .	5 UML	c	c	c	c	c	c	h	c	c	—	1—	7·5	T.
,, . .	5 UM	c	c	c	c	c	c	h	c	c	—		10·0	S.
,, . .	L	c	e	c	c	hk′	c	hk	e(e)	c	—	2—	7·0	B.
July . . .	U	e	e	e	e	h	e	h	e	e	—			
,, . . .	5 M	e	c	c	e	h	c	h	e	e	—	3—	7·5	T.S.B.
,, . . .	L	e	e	e	e	hk′	e	hk	e	e	—			
August . .	5 UM	c	c	c	c	c	c	h	c	c	—	2—	7·5	T.S.
,, . .	L	cc′	cc′	c	c	hk′	c	hk	c	c	—			
,, . .	5 U	a	a	a	a	a	a	h	a	a	—			
,, . .	M	a	a	a	a	e	a	h	a	a	—	1—	7·5	B.
,, . .	L	e	e	a	a	hk′	h	hk	e	e	—			
September	U	e	e	a	a	a	a	h	a	a	e			
,,	5 M	c	c	a	a	hk	a	hk	e	e	e	1 -	12·5	T.
,,	L	e	e	a	a	hk	c	hk	e	e	e			
,,	U	e	e	a	a	a	a	h	a	a	e			
,,	5 M	e	a	a	a	hk′	a	hk	a	a	e	1—	7·5	S.
,,	L	e	e	a	a	hk′	e	hk	e	e	e			
,,	U	a	a	a	a	a	a	h	a	a	e			
,,	5 M	e	a	a	a	hk′	c	hk	a	a	e	1 –	8·0	B.
,,	L	e	e	a	a	hk′	e	hk	e	e	e			
October .	5 U	e	e	a	a	a	a	h	a	a	e			
,, .	5 M	e	e	a	a	hk′	a	hk	e	e	e	3	7·5	T.S B.
,, .	L	e(e)	e	a	a	hk′	e	hk	e	e	e			
November	U	e	e	e	e	e	e	h	a	a				
,,	5 ML	e	e	a	a	hk′	a	hk	e	e		3	7·5	T.S.B.
December	U	e	e	e	e	e	e	h	e	e				
,,	5 M	e	e	cc′	e	h	e	h	e	e	—	2	7·5	T.S.
,,	L	ch′	ch′	cc′	e	h	h	h	e	e	—			
,,	5 U	e	e	c	e	e	c	h	e	e	—	1—	7·5	B.
,,	ML	ch′	eh′	c	e	h	c	h	e	e	—			
1926														
January .	U	e	e	a	a	e	a	g	a(e)	e	—			
,, .	6 ML	e	e	a	a	i	a	h	a(e)	e	—	3 –	7·5	T.S.B.

c′, g′, i′, k′ = walls. (e) — one twig.

Table VII (contd.)

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems	Size in cms.	Part of shrub
1926														
February.	U	i	i	a	a	a	c	g	a	a	—			
,, .	6 ML	i	i	a	a	ik′	a	h	c	c	—	1—	7·5	T.
,, .	U	e	e	e	c	e	e	g	a	a	—			
,, .	6 M	e	c	c	c	e	e	h	c	e	—	2—	6·0	S.B.
,, .	L	e	e	c	c	i	e	h	c	e	—			
March . .	U	e	e	e	e	c	g	g	g	e	—	2—	5·5	T.S.
,, . .	6 ML	i	i	c	c	ik′	c	ik	c	e	—			
,, . .	U	c	e	e	e	c	c	g	c	c	—			
,, . .	6 M	e	e	c	c	ik′	e	i	c	e	—	1—	7·5	B.
,, . .	L	e	e	c	c	ik′	c	i	c	c	—			
April . .	UM	e	c	e	e	e	e	g	c	c	c			
,, . .	6 L	eg′	eg′	c	c	g′	a	ik	c	c	c	2—	6·0	T.B.
,, . .	U	e	e	e	e	ec	e	g	c	c	—			
,, . .	6 M	e	e	a	a	ec	c	g	a	a	—	1—	6·0	S.
,, . .	L	i′	i′	a	a	i′	c	ik	c	c	—			
May . . .	U	Nat. Pink	c	c	c	c	c	g	c	c	—			
,, . . .	6 M		e	c	c	ci′	c	i	e	e	—	1—	7·5	T.
,, . . .	L		e	c	c	ci′	c	ik	e	e	—			
,, . . .	U	c	c	c	c	c	c	g	c	c	c			
,, . . .	6 M	e	e	e	c	ci′	e	i	c	e	c	2—	8·0	S.B.
,, . . .	L	e	e	c	c	ci′	c	ik	e	c	c			
First series		—	—	b	bz	bz	b	z	bz	bz				

e′, g′, i′, k′ = walls. (e) — one twig.

months November to April. This winter absence of lesser acidity might almost be said to start in October, where the epidermis is generally of pH 5·2—4·8. During the summer months, May to September, the greater acidity is either absent, or present in the lower rather than in the upper parts of the twigs.

This analysis of the variations applies also the sub-epidermis, where the more acid winter condition extends from November to April, and the less acid summer condition from May to September.

The *cortex* and *endodermis* vary in the same sense as each other. The range is pH 5·9—4·8, with the range of greater acidity dominant only during December in part, and the range of lesser acidity clearly dominant from May to October, with a partial exception in July.

The *pericycle* in the lower parts of the twigs was in general very acid, in the range pH 4·4—4·0. In the upper parts of the twigs pH 5·9 or pH 5·6 occurred from May to October, in December (part) and in February (part), with the acid July stems as an exception. The range pH 5·2—4·8 occurred in November, December (part), January, February (part), March and April. There is thus a general tendency in the pericycle of the upper parts of the twigs towards a more acid winter condition and a less acid summer condition.

In the *phloem* the variation is again considerable, a to e or occasionally h or g. The July stems are again exceptionally acid for the summer months. The range of greater acidity is shown by the upper parts of the twigs in November, December (part), February (part), March and April. Omitting the acid July twigs, the range of lesser acidity is shown in these parts during May to October.

March, April and July are the only months during which the range pH 5·2—4·8 occurs in the *pith* of the upper parts of the twigs. During the rest of the year the upper parts are in the range pH 5·9—5·6, with the lower parts usually more acid, pH 5·2—4·8.

The *epidermal hairs* were uniformly in the e range, where they were seen at all.

Rhododendron ponticum

This material was obtained from shrubs in the grounds of Queen's University. Sometimes two shrubs and a double set of twigs were used, but the results were very uniform.

The records are summarised in Table VIII. The *cortex*, *endodermis* and *pith* were uniformly of pH 5·2—4·8 throughout the year. The *cuticle*, where it was observed, was always in the range pH 4·4—4·0. The *xylem* was usually in the same range, with occasional cell walls of pH $<$ 3·4. The *sub-epidermis* was in the e range (pH 5·2—4·8) with the exception of the twigs for February (pH 4·4). The greater acidity occurred also in the *epidermis* in February and in some epidermal cells for April.

Table VIII. *Rhododendron ponticum*

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Cuticle	No. of stems	Size in cms.	Part of shrub
1925														
June . .	UM	e	e	e	e	o	o	h	e	o	—			
,, . . .	5 L	e	e	e	o	hk′	e	hk	o	o	—	3--	7.5	T.S.B.
July . .	UM	e	e	e	e	e	h	h	o	o	—			
,, . . .	5 L	e	e	e	c	hk′	h	hk	e	o	—	2—	10.0	T.S.
,, . . .	5 UML	e	e	e	e	hk	h	hk	c	o	—	1—	6.0	B.
August . .	5 UML	e	e	c	e	hk	h	h	e	c	—	3—	7.5	T.S.B.
September	U	e	e	e	e	c	c	h	e	c	—			
,,	5 ML	e	e	c	e	h	e	h	c	c	—	3 --	7.5	T.S.B.
October .	5 UML	e	e	e	e	ek	h	h	c	o	—	3—	7.5	T.S.B.
November	UM	e	e	e	e	h	h	h	c	e	—			
,,	5 L	e	e	e	c	hk	h	hk	e	c	—	3—	7.5	T.S.B.
December	5 UML	e	e	e	e	h	e	hk	c	e	—	3—	10.0	T.S.B.
1926														
January	6 UML	e	e	e	e	ik′	g	ik	e	o	i	3—	6.0	T.S.B.
February	6 UML	g	g	e	e	ik′	g	ik	c	o	h	3—	6.0	T.S.B.
March . .	6 UML	e	e	e	c	ik′	g	ik	c	o	h	3—	10.0	T.S.B.
April . .	U	o	e	c	e	g	g	g	c	e	—			
,, . .	6 ML	eg	e	e	c	ik	g	ik	c	c	--	2 –	7.5	T.B.
,, . .	U	e	e	e	e	g	c	g	c	e	—			
,, . .	6 ML	eg	e	e	c	ik	c	ik	c	e	—	1---	7.5	S.
May . . .	U	e	e	e	c	g	c	g	c	e	g			
,, . . .	6 ML	c	e	e	e	i	o	i	c	o	g	3—	5·5	T.S.B

k′ — walls.

The only tissues showing much variation were the pericycle and the phloem. The *pericycle* in the lower parts of the twigs was almost always rather acid, in the pH 4·4—4·0, or very acid, pH < 3·4, particularly the walls. The upper parts of the twigs had the pericycle of lesser acidity, pH 5·2—4·8, from June to October with July (part) and August as more acid. Again we get an indication of a tendency towards a more acid reaction of the pericycle during the winter.

The reaction of the *phloem* was always below pH 5·2, and was above pH 4·8 only in June, September, December, April (part) and May. No clear seasonal variations were found in this tissue.

Veronica sp. × *V. andersoni*

This material was derived from a hybrid growing in a sheltered position with a southern aspect in the Class Garden, Queen's University. The results are summarised in Table IX.

In this case the greater acidity of the lower parts of the twigs was so distinct in several of the tissues that it was thought advisable to extract the records for that part and to group the upper and middle parts together as in Table IX.

The *xylem* in the upper and middle parts is of pH 4·4—4·0 or practically restricted to pH 4·4 with the use of bromo-cresol green. In the lower part it is usually more acid, in part (**h k**) or as whole (**i k**). The first range is pH 4·4—4·0 or pH < 3·4; the second is pH 4·0 or pH < 3·4, so that altogether the xylem of the lower part is distinctly more acid than the xylem of the upper and middle parts.

The *central pith* is more acid in the lower part of the twig in February, March and April, and more acid groups of cells appear in the *pericycle* in the first two months.

The majority of the cells from the epidermis to the phloem were more acid in the lower parts of the twigs for April (part), May (part) and June.

Considering only the upper and middle parts the *epidermis* and *sub-epidermis* were of pH 5·6 in June, July, September and October, also one twig in November, one each in March, April and May. At other times the range was pH 5·2—4·8, making the winter range more acid and the summer range less acid in these tissues, with some exceptions.

The reaction of the *cortex* and *endodermis* was predominantly less acid from March to October, more acid from November to February, with August as an exception in the case of the endodermis. The *pericycle* and *phloem* showed similar winter and summer ranges, with August a more acid exception as in the endodermis, and the winter more acid range extended to include March, the summer range being from April to October with greater acidity appearing in both May and August in the case of the phloem.

Table IX. *Veronica* sp. × *V. andersoni*

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems Size in cms.	Part of stem
1925													
June . .	5 UM	c	c	c	c	c	c	h	e	c¹)	–	3 · 7·5	T.S.B.
July . .	5 UM	c	c	c	c	c	c	h	c	c	c	3--6·0	T.S.B.
August. .	5 UM	e	e	c	e	e	e	h	e	e	e	2 6·0	T.S.
„ . .	5 UM	e	e	e	e	e	c	h	e	e	c	1—6·0	B.
September	5 U	c	c	a	a	a	c	h	c	a	c	1—6·0	T.
„	M	c	c	a	c	c	c	h	c	c	e		
„	5 U	c	c	a	a	c	c	h	c	a	c	2—6·0	S.B.
„	M	c	c	a	c	c	c	h	c	a	c		
October .	5 UM	c	c	c	c	c	c	h	c	c	c	1—6·5	T.
„ .	5 U	c	c	c	c	c	c	h	c	c	c		
„ .	M	c	c	c	c	c	c	h	c	a	c	2—6·0	S.B.
November	5 UM	e	e	c	e	e	e	h	e	e	e	1—7·5	T.
„	5 UM	c	c	c	c	c	c	h	c	c	c	1-- 7·5	S.
„	5 UM	e	e	c	e	e	c	h	e	e	c	1 7·5	B.
December	5 UM	e	c	c	e	e	e	h	c	e	c	3--8·0	T.S.B.
1926													
January .	6 UM	e	c	c	c	c	c	g	c	e	c	2- 6·0	T.S.
„ . .	6 UM	c	e	c	e	c	c	g	e	c	e	1—6·0	B.
February.	6 UM	e	e	e	c	c	c	g	e	c	e	3—6·0	T.S.B.
March . .	6 UM	e	e	c	c	e	e	g	c	c	c	1—6·0	T.
„ . .	6 UM	c	e	c	c	e	c	g	c	c	c	1—6·0	B.
„ . .	6 UM	c	c	c	c	·c	c	g	c	c	c	1—6·0	S.
April . .	6 UM	e	e	c	c	c	c	g	c	c	c	2—6·0	T.S.
„ . .	6 U	e	e	c	c	c	c	g	c	c	c	1--6·0	B.
„ . .	M	c	c	c	c	c	c	ik	c	c	c		
May . . .	6 UM	c	c	c	c	c	c	g	c	c	c	1 8·0	T.
„ . . .	6 U	e	e	e	c	c	c	g	c	c	c	1 8·0	S.
„ . . .	M	e	e	e	h	c	c	g	c	c	e		
„ . . .	6 UM	e	e	c	c	c	c	g	e	c	c	1 8·0	B.
1925													
June . .	5 L	e	c	e	e	c	c	hk	c	c	–	3	
July . .	5 L	c	c	c	c	c²	c	hk	c	c	c	3	

1) a in TOP twig. 2) h in Base twig. CUTICLE i or e.

Table IX (contd.)

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray	Pith, central	Hairs	No. of stems Size in cms.	Part of stem
1925													
August. .	5 L	e	e	e	e	e	e	hk	e	e	e	2	
,, . .	5 L	e	e	e	e	e	e	hk	e	e	e	1	
September	5 L	e	e	a	e	e	e	h	e	a	e	1	
,,	5 L	e	a	a	a	e	e	h	e	a	e	2	
October .	5 L	e	e	e	e	hk	e	hk	e	e	e	1	
,, .	5 L	e	e	e	e	e	e	hk	e	a	e	2	
November	2 L	e	e	e	e	e	e	hk	e	a	e	1	
,,	5 L	e	e	e	e	e	e	h	e	e	e	1	
,,	5 L	e	e	e	e	e	e	h	e	e	e	1	
December	5 L	e	e	e	e	e	e	h	e	e	e	3	
1926													
January .	6 L	e	e	e	e	e	e	g	e	e	e	2	
,, .	6 L	e	e	e	e	e	e	g	e	e	e	1	
February	6 L	e	e	e	e	e (i)	e	ik	e	e	e	3	
March . .	6 L	e	e	e	e	e (i)	e	ik	e	e	e	1	
,, . .	6 L	e	e	e	e	e (i)	e	ik	e	e	e	1	
,, . .	6 L	e	e	e	e	e (g)	e	h	e	e	e	1	
April . .	6 L	e	e	e	e	e	e	ik	e	e	e	1	
,, . .	6 L	e	e	e	e	e	e	ik	e	e	e	1	
,, . .	6 L	e	e	e	e	e	e	ik	e	e	e	1	
May . . .	6 L	e	e	e	e	e	e	ik	e	e	e	1	
,, . . .	6 L	e	e	e	e	e	e	g	e	e	e	1	
,, . . .	6 L	e	e	e	e	e	e	ik	e	e	e	1	

(i) (g) — groups of cells.

The *outer pith* is very similar to the phloem throughout, but shows pH 5·9 instead of pH 5·6 in the autumn. Greater acidity occurs in the *central pith* from November to January, and also in May and August, so that in this tissue the winter condition is of shorter duration. The *epidermal hairs* were uniformly of pH 5·2—4·8.

Considering the lower parts of the twigs we find much the same seasonal variation, except in those months where the lower parts are more acid than the upper and middle parts of the twigs. Because of this other phenomenon the range pH 5·2—4·8 tends to predominate in most tissues throughout the year. July and September are the only two months showing lesser acidity in most of the tissues, with also one side twig in November and one side twig in March.

Viburnum tinus

This material was obtained from a bush in a sheltered position in the Research Garden, Queen's University. The results are summarised in Table X.

Again we find the *xylem* walls about pH 4·4 in the upper parts and about pH 4·0 or pH $<$ 3·4 in the lower parts of the twigs. The *pericycle* also shows this same kind of variation.

With the exception of the basal twig for September (pH 5·9) and parts of the top twig for May (pH 4·4), the *epidermis* is uniformly in the range pH 5·2—4·8. The same September twig and groups of cells at pH 5·6 in July form the only exceptions to pH 5·2—4·8 for the *sub-epidermis.*

The *cortex* is mainly in the same e range; exceptions being odd cells in the July twigs, two of the three April twigs and the middle part of the basal twig for May, which were all of pH 5·6. The basal twig for September was less acid, pH 5·9. The *endodermis* was nearly similar, being of pH 5·6 in July and for the top and side April twigs, as well as for the basal September twig.

The *pericycle*, apart from the acid, **h k** or **i k**, lower parts, was usually in the common range pH 5·2—4·8 and showed no seasonal variation.

The *phloem* was mainly in the range pH 5·2—4·4; the one exception being the relatively alkaline basal twig for September. It is clear that a solitary variation of this character requires re-investigation. It should be noted that nearly all the tissues of this twig, upper, middle and lower parts are recorded as of an abnormally low degree of acidity. Other similar cases occur and we view these records with suspicion. The reaction may actually be abnormal for some unexplained reason, or something abnormal may have happened during the process of determining the reaction. Absolute cleanliness is a very necessary condition for succes with the Range Indicator Method. A new set of watch-glasses, a badly washed razor, a suspicion of free alkali or even

Table X. *Viburnum tinus*

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray and central	Hairs	Cuticle	No. of stems	Size in cms.	Part of shrub
1925														
June . .	5 UML	e	e	e	e	e	e	h	e	hk		1	5·0	T.
,, . .	UM	e	e	e	e	e	e	h	e	hk		2	5·0	S.B.
,, . .	5 L	e	e	e	e	hk	h	h	e	hk				
July . .	U	e	e	e	e	e	e	h	e	e				
,, . .	5 M	e	ee	ee	e	e	e	h	e	e		3	6·5	T.S.B.
,, . .	L	e	ee	ee	e	hk	e	hk	e	e				
August . .	U	e	e	e	e	e	e	h	e	hk		3	6·5	T.S.B.
,, . .	5 ML	e	e	e	e	hk	e	hk	e	hk				
September	UM	e	e	e	e	h	e	h	e	k		1	7·5	T.
,,	5 L	e	e	e	e	hk	e	hk	e	k				
,,	5 U	e	e	e	e	h	e	h	e	k		1	7·5	S.
,,	M	e	e	e	e	h	e	h	ee	k				
,,	L	e	e	e	e	hk	e	hk	e	k				
,,	5 U	a	a	a	a	a	a	h	a	e		1	7·5	B.
,,	M	a	a	a	a	a	a	h	ae	e				
,,	L	a	a	a	a	hk	a	hk	ae	e				
October .	5 U	(1)	(1)	e	e	e	e	h	e	e		3	7·5	T.S.B.
,, .	ML	(1)	(1)	e	e	hk	e	hk	e	e				
November	U	e	e	e	e	e	e	hk	e	h		3	5·0	T.S.B.
,, .	5 ML	e	e	e	e	hk	e	hk	e	h				
December	U	e	e	e	e	e	e	h	e	h	k	3	10·0	T.S.B.
,, .	5 ML	e	e	e	e	hk	e	hk	e	h	k			
1926														
January .	U	e	e	e	e		e	g	e	i	i	3	6·5	T.S.B.
,, .	6 ML	e	e	e	e	i	e	h	e	i	i			
February	U	e	e	e	e	-	e	g	e	i	i	3	6·5	T.S.B.
,, .	6 ML	e	e	e	e	i	e	h	e	i	i			
March . .	U	e	e	e	e	i	e	g	e	i	k	3	10·0	T.S.B.
,, . .	6 ML	e	e	e	e	i	g	h	e	i	k			
April . .	U	e	e	e	e	e	g	g	e	i	i			
,, . .	6 M	e	e	e	e	ik	g	ik	e	i	i	1	6·5	T.
,, . .	L	e	e	e	e	ik	g	ik	eg	i	i			

(1) natural pink.

Table X (contd.)

Date	Indicators Part	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith, ray and central	Hairs	Cuticle	No. of stems	Size in cms.	Part of shrub
1926														
April . .	6 U	e	e	e	e	e	e	g	e	i	i			
,, . .	M	e	e	e	e	ik	g	ik	e	i	i	1—	5·0	S.
,, . .	L	e	e	e	e	ik	g	ik	eg	i	i			
,, . .	6 U	e	e	e	e	e	e	g	e	i	i	1—	5·0	B.
,, . .	M	e	e	e	e	i	g	ik	e	i	i			
,, . .	L	e	e	e	e	ik	g	ik	eg	i	i			
May . . .	U	e	e	e	e	e	e	g	e	eg	i			
,, . . .	6 M	g	e	e	e	i	g	i	e	eg	i	1—	10·0	T.
,, . . .	L	g	e	e	e	ik	g	ik	e	eg	i			
,, . . .	U	e	e	e	e	e	e	g	e	eg	i	1—	11·5	S.
,, . . .	6 M	e	e	e	e	g	e	g	e	eg	i			
,, . . .	L	e	e	e	e	ik	e	ik	e	eg	i			
,, . . .	U	e	e	e	e	e	e	g	e	eg	i			
,, . . .	6 M	e	e	e	e	e	e	g	e	eg	i	1—	11·5	B.
,, . . .	L	e	e	e	e	ik	e	ik	e	eg	i			

a rather heavy sigh with its accompanying exhalation of carbon dioxide may spoil a whole series of sections. The wonder is not that we do come across abnormal results in an analysis of this extent, but that the reaction of the tissues can be demonstrated in any case as being in exactly the same range throughout the year.

Reverting to the variation shown by the *phloem* within the range pH 5·2—4·4, the lower range pH 4·4 occurs only in one or both of the two lower parts of all twigs in March and April, in one twig in May and in two twigs in June. There is thus an indication of a summer more acid condition of the phloem in this case, with pH 5·2—4·8 more or less constant during the rest of the year.

The *pith*, apart from the abnormal September twig, shows pH 5·2—4·8 throughout the year, with pH 4·4 only in some cells in the lower part of the April twigs, and some cells of pH 5·6 in the middle part of the side September twig.

The *hairs* in this stem show considerable variation. Omitting the one abnormal September twig, the range is pH 5·2 to pH $<$ 3·4. The higher range, pH 5·2—4·8 appears only in some of the hairs in May and in all of them in July and October. No clear seasonal variation can be traced, although there is some indication of a short, less acid, summer period. The *cuticle,* where observed, was uniformly of pH 4·0 or pH $<$ 3·4.

DISCUSSION

The present series of records as summarised in Tables I—X differs from those given for the sunflower (Chapter XII) and the bean (Chapter XIII). These latter two are annual plants which show some changes in tissue reactions at special stages in their life-periods, such as at germination, during lignification, when maturing as full-grown flowering plants and so on. The present series deals either with stems which survive the winter as in the woody plants used, as also in the hairy chickweed and wallflower, or with stems which are renewed in one way or another throughout the year as in the deadnettle and groundsel.

In the former cases we can distinguish the stages of lignification and maturing, particularly in the woody stems, as reaction differences between the upper, middle and lower parts of the stems. This aspect has already been mentioned in connection with most species and it is only necessary to point out that, in general, maturing changes in stem tissues seem to involve increases in the actual acidity of the tissues. Many exceptions to this tendency and some records in the opposite sense, where the lower parts are *less* acid than the upper parts, all show that this must be taken merely as a general indication and not as a rule. Much more work of this kind is required before we can say we really know the reaction changes which are the cause or result of maturing changes in stem tissues.

As yet the normal concomitance of lignification and increasing acidity and also the concomitance of maturing chlorenchyma and decreasing acidity are about the only general phenomena of reaction changes which are related in a reasonable way to the rest of our knowledge of plant physiology. In the former case a causal relation is far from being demonstrated. In the latter case, although the increased using up of the carbon dioxide of respiration seems a plausible explanation, the decreased actual acidity recorded for mature chlorenchyma should firstly be taken with reserve on account on the difficulty of determining even the colours given by the indicators in green tissues and secondly be regarded as possibly connected with buffer changes and/or the general carbon dioxide balance or with metabolic effects other than simple photo-synthesis of respiratory carbon dioxide.

Apart from these changes due to maturing, the present records show clearly that in some stem tissues of some of the plants examined the hydrion concentration remains remarkably constant throughout the year. Variations, usually to a slight extent, occur which do not seem to be related to the season or to stages in the life of the plant; and in a few cases unexplained fluctuations of considerable extent do occur.

Other variations are recorded which appear in some successive months of the year and not during the rest of the year. These, whether related to metabolic changes or not, are for present purposes described as "seasonal". The main seasonal variations have been summarised in Table XI, the acidity being marked according to whether it is greater (plus) or less (minus) during the months named in the following column. The plus and minus signs are therefore no indication of the actual hydrion concentration, but indicate only whether the actual acidity is greater or less than during the rest of the year. During the periods given there may or may not be some slight variation in one or more of the stems or parts of the stems used for each month. The periods are, therefore, to be taken as indicating general tendencies, with months which are clearly exceptions given in brackets or otherwise.

In Table XI U. M. and L. are used in the first column where the seasonal variation applies only to one or two parts of the stems.

Considering the seasonal variations found, hairs both in *Cerastium* and *Viburnum* are less acid in October, while in *Senecio* they are *more* acid in January and February. Superficially the lesser acidity of the hairs in *Cheiranthus* (shade) for November and December seems to be similar to the first records, but it should be pointed out that the hairs in the sun plants were uniformly of pH 5·9, while those of the shade plants from June to October were more acid, pH 5·2—4·8 and attained pH 5·9 in November and December. One might have expected the *summer* condition of the shade plants to resemble the condition in the sun plants,

Table XI. *Seasonal summary for stems (to show general tendencies)*

Plant	Tissues	Winter Changes		Summer Changes	
		Acidity	Periods	Acidity	Periods
Cerastium tomentosum	Hairs	—	October		
	Epidermis	+	October—May	—	July-September
	Epidermis	+ +	January		
	Cortex	+	Februar—March		
	Phloem	VV\	Erratic Nov.—Jany.	+	July
Cheiranthus cheiri (Sun)	Sub-Epid. Cortex	+	I Plant October		
	Endodermis	+	October		
	Endodermis	+ +	Nov.—Jany.		
	Phloem Pith,central	+	December		
Cheiranthus cheiri L.— (Shade) L.—	Hairs	—	Nov.—Dec.		
	Epidermis			+	June—July
	Endodermis	+	Nov.—Feby.		
Lamium purpureum L.—	Sub-Epid.	—	Dec.—March	+	June
	Sub-Epid.			—	July
	Pith, ray			+	July-October
Senecio vulgaris	Hairs	+	Jany.—Feby.		
	Epidermis	—	Dec. and Feby.	+	M. L. May
	Pith, ray	+	Sept. and Nov. Jany.—March		
Aucuba japoponica	Epidermis	—	Aug.—Feby. (April)	+	(March) May—July
	Epidermis	+	December		
	Sub-Epid. Cortex Endodermis	+	December (Oct.)	+	(April)May - June
	Pericycle Phloem	+	December	+	April June (part)
Ligustrum vulgare U. M. chiefly	Epidermis Sub-Epid.	+	Oct.—April	—	June-September
	Cortex Endodermis	+	December	—	May—October
	Pericycle	+	Nov.—April	+	July
	Phloem	+	Nov.—April	+	July
	Phloem			—	May—October
	Pith	—	Aug.—Feby.	+	March—July

+ = More acidity. — = Less acidity.

Table XI (contd.)

Plant	Tissues	Winter Changes		Summer Changes	
		Acidity	Periods	Acidity	Periods
Rhododendron ponticum	Epidermis Sub-Epid.	+	Februayr	—	
U. M.	Pericycle	+	(August) Nov.—May	—	June—October
Veronica hybrid	Epidermis Sub-Epid.	+	Nov.—May	—	June—July Sept.—Oct.
	Cortex Endodermis	+	Nov.—Feby.	—	March—July Sept.—Oct.
U. M.	Pericycle	+	Nov.—March	—	April—July Sept.—Oct.
	Phloem Pith, ray	+	Nov.—March (May and Aug.)	—	June—July Sept.—Oct.
	Pith, central	+	Nov.—Jany. (May and Aug.)	—	February—July Sept.—Oct.
	(Most)			—	July. Sept.-Oct.
Viburnum tinus	Hairs	—	October	—	May and July
M. L.	Cortex Endodermis			—	April—July
	Phloem			+	March—June
	Pith			+	April

+ = More acidity. — = Less acidity.

but here we get the reaction of the hairs in the shade plants during the last two months of the year the same as that in the sun plants throughout the year. Further investigation of this point is clearly suggested. One might theorise glibly, as with other reaction phenomena, but we do not know; and the more acid winter condition of the hairs of *Senecio* introduces a complication which forbids generalisations.

The epidermis in *Senecio* and *Aucuba* and the sub-epidermis in *Lamium* and the pith in *Ligustrum* are the only other tissues showing a less acid winter condition. It should be noted that the only tissue internal to the sub-epidermis which shows lesser acidity in winter is the pith of the privet. In *Lamium* the lesser acidity also occurs in July (for the sub-epidermis), so that it is not confined to the winter. This summer variation may be connected with the main flowering period, as also may the increased acidity in June.

Apart from these outer tissues, all the winter variations are in the direction of increased acidity. Decreased photosynthetic activity is the facile explanation of this tendency but it is more probable that the true explanation is complex and probably involves the separate consideration of each tissue and possibly of each species. It may, however, be noted that the tissues showing this increased acidity include the cortex, endodermis and phloem in four of the five shrubs and two of the herbaceous species examined; the epidermis in three shrubs and one herb; and the sub-epidermis in four shrubs. The pith is clearly less subject to seasonal changes than are the outer tissues like the epidermis, sub-epidermis and cortex, or the inner but more active cells of the phloem.

The summer changes are more varied in their direction. Amongst the herbs such changes as occur may be connected in some still obscure way with the flowering period, but the extended period of increased acidity in the outer pith of *Lamium* seems more probably a maturing change involving lignification of the walls of that tissue. The other relatively long period of lesser acidity in the epidermis of *Cerastium* is complementary to the winter period of increased acidity which reaches a maximum in January.

Amongst the shrubs the *Veronica* records, when one eliminates the lower part of the stem, show very clearly two seasons. One a winter period of greater acidity and the other a longer or shorter summer period of lesser acidity, with August as an exception to the summer range. In the lower part of the *Veronica* stems the summer period of lesser acidity is reduced to one month (July) for most of the tissues showing any seasonal change.

In *Viburnum* the cortex and endodermis show a short summer period of lesser acidity and this species might be added to the other four shrubs showing a greater acidity in winter, but the lesser acidity occurs sporadically during the summer months, in April, June and July (see Table X).

The phloem shows greater acidity for a short period during the summer in *Cerastium*, *Aucuba*, *Ligustrum* and *Viburnum*. As already suggested this *may be* connected with the flowering period, but two of the shrubs are kept trimmed and do not flower, so that if there is any connection it would appear to be with preparations for flowering or with changes concomitant with the flowering period rather than a cause or effect of anthesis.

The pericycle in *Rhododendron* and *Veronica* has a more acid winter period with a complementary less acid summer period. But in *Ligustrum* and *Aucuba* a more acid winter period is shown, together with a short more acid period in July or during April to June.

CONCLUSIONS

The data obtained enable us to draw a few limited conclusions concerning the hydrion concentration of the stem tissues *in the plants examined*. Further, as these stems represent herbaceous and woody types, also relatively less acid types such as *Cheiranthus* and *Aucuba*, and more acid types such as *Rhododendron* and *Viburnum*, we might expect any general indications to be more or less applicable to other cases. We have, however, no data of the seasonal changes, if any, in the acid type of herbaceous stem such as occurs in the Polygonaceae and Geraniaceae (see Chapter X).

Our conclusions may be summarised as follows — IN THE PLANTS EXAMINED —

I. The hydrion concentration of the inner tissues pith and xylem tends to remain constant throughout the year or to fluctuate only slightly without any obvious correlation beween the changes and the seasons.
2. The hydrion concentration of the cortex, endodermis and phloem tends to increase during the winter or decrease during the summer in most species.
3. The epidermis and sub-epidermis, particularly in the woody plants examined, tend towards greater acidity during some part of the winter period.
4. The seasonal variations normally affect the outer tissues much more than the inner tissues.
5. Each tissue and each species should be considered as an individual case when any explanation of the mechanism of these variations is considered.
6. Apart from the seasonal changes a general increase of acidity is found to be concomitant with lignification and similar processes which take place in the lower parts of the stems, especially in the shrubby plants.

That such conclusions are reasonable deductions from the data obtained seems to us quite clear. That such conclusions are possible seems to us to furnish further proof of the value of the Range Indicator Method as a practical process for the determination of the hydrion concentration of plant tissues, perhaps with a lesser degree of accuracy but with a greater degree of certainty than is possible with the many other methods which have been reviewed above, Part II. It is true that what is determined by this method is the range within which the actual reaction lies, but it is also true that variations occur which bring the hydrion concentration out of one range and into another. The variations which occur under natural conditions are, in fact, so large that for the purpose of a general survey of the actual acidity of plant tissues the Range Indicator Method is distinctly useful.

SUMMARY

1. The Range Indicator Method has been applied to the determination of the hydrion concentration of the stem tissues of selected herbaceous and woody plants throughout the year.
2. A striking uniformity of pH has been found in several cases, see Tables II, III, IV, VIII and X.
3. Variations have been observed, and these may be grouped as changes with maturing, seasonal changes and fluctuations.
4. Maturing changes show from upper to lower parts of the stems and are usually in the direction of increased acidity.
5. Seasonal changes occur in the outer rather than in the inner tissues.
6. For general deductions the reader is referred to the section headed "Conclusions".

Comparing these results with those of Abbott on apple and peach tips (fig. 17);

1. July is seen to be a summer month when the tissues are frequently more acid; 2. the herbaceous stems examined, together with those of *Aucuba* and *Ligustrum*, show various periods of increased actual acidity, like those in the apple but longer in duration and usually later in the year; 3. in *Rhododendron* and *Veronica* stems there is a short acid period in August and a longer acid period covering November to January or later to May; this acid winter condition contrasts with the high winter pH values in both apple and peach; 4. the data for the juice again

appear to be averages for all the tissues as in *Bryophyllum* but, of course, the plants examined are different and close comparison is not possible. It should be noted that apple and peach belong to Rosaceae, an "acid" family, which might be better compared with *Rhododendron* than with any of the other species examined by the R.I.M.

(b) *Gymnosperm Leaves.*

Doyle and Clinch (1926 p. 232) using Atkins' drop indicator method, reported a relatively constant pH for the pressed juice of conifer leaves, thus —

Seasonal Values of pH of Conifer Leaves

	1925							1926
	May 11	June 12	July 31	Aug. 24	Sept. 25	Oct. 22	Nov. 28	Jan. 13
Austrian Pine	3·8	3·7	3·9	3·5	3·7	3·6	3·6	3·9
Pinus montana	—	—	—	3·6	—	3·8	—	—
Tsuga	3·5	3·2	3·3	3·5	3·5	3·5	3·6	3·5
Abies	3·7	3·5	3·7	3·5	3·8	3·7	4·3	3·7
Sitka Spruce.	3·5	3·4	3·5	3·7	3·7	3·7	3·7	3·7
Cupressus . .	—	4·6	4·6	5·1	5·1	5·0	4·8	5·1
Juniperus . .	—	4·6	4·6	5·2	5·3	5·4	5·3	5·3
Larch (Eur.)	3·3	3·5	3·8	4·1	3·8	4·3	fallen	fallen
Douglas Fir .	—	—	—	3·7	—	—	—	—

These records show a variation, but, considering the method, it would be difficult to trace a seasonal significance in the changes.

(c) *Angiosperm Leaves*

The tissues of the leaves of several herbaceous and shrubby plants were investigated at monthly intervals throughout the year by Rea and Small. The methods were as described for stems, the upper, middle, and basal part of each leaf being sectioned. In the case of shrubs the end leaf from a twig on the top, side and base of each shrub was taken; this is indicated by T. S. B. in the tables below. The results are here published for the first time and are summarised below in Tables XII—XXI.

Table XII. *Dianthus caryophyllus var.*

Date	Indicators Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Fibres	Bundle sheath	Guard cells	Lower epidermis	No. of leaves	Size in cms.
1925												
June	5 UM	c	a	a	h	c	h	a	a	a	3	4·3
,, ...	L	c	a	a	h	c	hk	a	a	a		
,, ...	UML	c	a	a	h	c	h	a	c	c	2	5·0
July	5 U	c	c	a	h	c	h	a	c	c		
,, ...	M	e	c	a	h	c	h	a	c	c	1	5·0
,, ...	L	c	c	a	h	c	h	a	c	c		
August . . .	5 UML	c	a	a	h	a	h	a	c	c	3	6·2
September .	5 UM	c	c	a	h	a	hk	a	a	a	3	6·2
,, .	L	c	c	a	h	a	c	a	a	a		
October. . .	5 UML	c	c	c	h	c	hk	c	c	c	3	6·2
November. .	5 UM	c¹)a¹)	a	a	h	c	hk	a	a	a	(2)	
,, . .	L	a	a	a	h	c	c	a	a	a	1	7·5
,, . .	U	c	a	a	h	a	hk	a	a	a		6·2
,, . .	M	ca	a	a	h	a	hk	a	a	a	2	5
,, . .	L	ca	a	a	h	a	c	a	a	a		
December. .	5 UML	e	c	c	h	e	h	—	c	c	1	7·5
,, . .	U	c	a	a	h	a	h	—	a	a		
,, . .	ML	a	a	a	h	a	h	—	a	a	1	7·5
1926												
January . .	6 U	a	a	a	g	a	hk	a	a	a		7·5
,, . .	M	e	a	a	g	a	hk	a	c	c	3	5
,, . .	L	c	c	c	g	a	hk	c	c	c		7·5
February . .	6 U	c	c	c	g	c	hk	c	c	c		
,, . .	M	c	c	c	g	c	hk	c	c	c	3	6·2
,, . .	L	c	c	c	g	c	hk	c	c	c		5
March . . .	6 UML	c	c	c	g	c	h	c	c	c	3	5
April	6 UM	c	a	a	g	c	hk	a	c	c		7·5
,, ...	L	c	c	c	g	c	c	c	c	c	2	5
,, ...	U	c	c	c	g	c	c	c	c	c	1	5
,, ...	ML	c	c	c	g	c	c	c	c	c		
May	6 U	c	c	a	g	c	gk	a	c	c		
,, ...	M	c	c	c	g	c	gk	c	c	c	3	7 5
,, ...	L	c	c	c	g	c	c	c	c	c		

1) a¹ applies to middle and c¹ to tip of leaf.

(2) an older leaf. Cuticle **e** throughout.

Table XIII.

Saxifraga umbrosa.

Date	Indicators Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Bundle sheath	Guard cells	Lower epidermis	No. of leaves	Size in cms.
1925											
June	5 UML	h	h	h	h	h	h	h	h	3	4·3 3·7 3·7
July	5 UML	h	h	h	h	h	h	h	h	3	3·7 3·7 3·1
August . . .	5 UML	h	h	h	h	h	h	h	h	3	3·7
September .	5 UML	h	h	h	h	h	h	h	h	3	3·7
October . . .	5 UML	h	h	h	h	h	h	h	h	3	3·1 2·5 3·1
November. .	5 UML	h	h	h	h	h	h	h	h	3	3·7 5·5
December. .	5 UML	h	h	h	h	h	h	h	h	3	3·7
1926											
January . .	6 UML	g	g	g	g	g	g	g	g	3	3·7 3·7 4·3
February . .	6 UML	g	g	g	g	g	g	g	g	3	2·5 5·5
March . . .	6 U	i	g	g	g	g	g	g	g	3	2·5 2·5 3·7
,, . . .	ML	g	g	g	g	g	g	g	g		
April	6 U	g	gi 1)	gi 1)	g	g	g	g	g	3	3·7
,, ...	ML	i	gi	gi	g	g	g	g	g		
May	6 U	gi 1)	g	g	g	g	c	g	g	3	4·3
,, ...	M	gi	gi	gi	g	g	c	c	c		
,, ...	L	gi	gi	gi	g	g	c	c	c		

1) pH 4·4 with spots of pH 4·0; hairs pH 4·4 or pH 4·1.
Leaves were both young and old.

Table XIV

Limnanthes Douglasii

BASES OF LEAFLETS

Date	Indicators	Position of leaflet on leaf	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Bundle sheath	Guard cells	Lower epidermis	No. of leaflets
1925											
June	5	UML	a	a	a	h	a	a	a	a	3
July	5	UML	a	a	a	h	a	a	a	a	3
August . . .	5	UML	a	a	a	h	a	a	a	a	3
September .	5	UML	a	a	a	h	a	a	a	a	3
October. . .	5	UML	a	a	a	h	a	a	a	a	3
November. .	5	UML	c	c	c	h	c	c	c	c	3
December. .	5	UML	a	a	a	h	a	a	a	a	3
1926											
January . .	6	UML	a	a	a	g	a	a	a	a	3
February . .	6	UML	a	a	a	g	a	a	a	a	3
March . . .	6	UML	a	a	a	g	a	a	a	a	3
April	6	UML	a	a	a	g	a	a	c	c	3
May	6	UML	c	c	c	g	c	c	c	c	3

PETIOLES

Date	Indicators	Epidermis	Subepidermis	Cortex	Endodermis	Pericycle	Xylem	Phloem	Ground tissue	No. of petiole
1925										
June	5	a	a	a	a	a	h	a	c	3
July	5	a	a	a	a	a	h	a	a	2
,, ...	5	a	a	a	a	a	h	a	c	2
August	5	a	a	a	a	a	h	a	a	2
,, ...	5	c	c	c	c	c	h	c	c	1
September	5	c	a	a	a	a	h	a	a	3
October	5	a	a	a	a	a	h	a	a	3
November	5	c	a	a	a	a	h	a	a	2
,, ...	5	c	a	a	a	a	h	a	c	1
December	5	c	a	a	a	a	h	c	c	3

Table XIV (contd.)

Date	Indicators	Epidermis	Subepidermis	Cortex	Endodermis	Pericycle	Xylem	Phloem	Ground tissue	No. of petiole
1926										
January	6	a	a	a	a	a	g	a	o	2
,, ...	6	a	a	a	a	a	g	a	o	1
February	6	c	a	a	a	a	g	a	a	3
March	6	c	a	a	a	a	g	a	a	3
April	6	o	a	a	a	a	g	a	c	2
,, ...	6	c	a	a	a	a	g	a	c	1
May	6	c	a	ac	a	a	g	a	o	3

Table XV
Primula vulgaris

Date	Indicators Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Fibres	Bundle sheath	Guard cells	Lower epidermis	No. of leaves	Size in cms.
1925												
June . . .	5 U	c	a	a	h	a	—	—	c	c	3	3·7 3·7 4·3
,, . . .	M	c	c	c	h	o	h	c	c	c		
,, . . .	L	c	c	c	h	e	h	c	c	c		
July . . .	5 U	c	c	c	h	c	—	—	c	c	c	
,, . . .	M	o	c	c	h	e	h	e	c	c	3	7·5 7·5 8·7
,, . . .	L	e	(e)c	c	h	e	h	e	e	e		
August . .	5 U	c	c	a(c)	h	c	—	—				
,, . .	ML	c	c	a(c)	h	c	h	c	c	c	2	6·2
,, . .	5 U	e	c	c	h	e	—	—	e	c	1	6·2
,, . .	ML	e	a(e)	a(e)	h	e	e	e	e	c		
,, . .	U	a	a	a	h	a	—	—	a	a		

(e) walls; (e′) spots; (c) walls; (c′) spots; c^2 in one leaf only; hairs and glands e throughout; cuticle e or g.

Table XV (contd.)

Date	Indicators Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Fibres	Bundle sheath	Guard cells	Lower epidermis	No. of leaves	Size in cms.
1925 September	5 M	a	a	a	h	c	c	c	a	a	3	10 7·5 10
,,	L	a	a(c′)	a(c′)	h	c	h	c	a	a		
,,	U	a	a	(c′)	h	a	—	—	a	a		
October. .	5 M	a	a(c′)	a(e′)	h	e	h	c	a	a	3	3·7 7·5 3·1
,, . .	L	a(c)	a(c)	a(′)	h	e	h	c	a	a		
,, .	U	c	c	c	h	h	—	—	c	c		
November.	ML	c	a(c′)	a(e′)	h	h	h	c	c	c	3	3·7 5·5
December	5 UM	a	a	a	h	e	—	a	—	a		
,,	L	a	a	a	h	c	—	c	—	a	2	7·5
,,	5 U	a	a	a	h	c	—	a	—	a		
,,	ML	a	a(c)	a(c)	h	e	—	c	—	a	1	7·5
1926 January .	6 UL	c	c	c	g	e	—	e	e	e		
,, .	M	e	e	cc_2	g	e	—	c	e	e	3	5·5 4·3
February .	6 U	e	e	e	g	e	—	e	e	e	3	6·2
,, .	ML	e	e	e	g	e	c	e	e	e		6·2 5·
March . .	6 U	e	e	e	g	c	c	e	e	c	3	3·7
,, . .	ML	c	e	e	g	e	e	c	e	c		
April . . .	6 U	c	a	a	g	c	—	—	c	c	3	4·3 4·3 3·7
,, . . .	M	c	a	a(c)	g	c	c	c	c	c		
,, . . .	L	c	a	a(c)	g	c	c	c	c	c		
May . . .	6 U	a	a	a(c)	g	a	a	a	a	a	3	7·5
,, . . .	ML	c	ac	ac	g	c	c	a	c	c		

(e) walls; (e′) spots; (c) walls (c′) spots; c^2 in one leaf only; hairs and glands e troughout; cuticle e or g.

Table XVI. *Aucuba japonica*

Date	Indicators / Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Bundle sheath	Guard cells	Lower epidermis	No. of leaves	Position on shrub	Size in cms.
1925												
June . .	5 U	c	c	c	e	c	a	c	c	1	T	5·6
,, . .	ML	c	c	c	h	c	a	c	c			
,, . .	5 U	e	c	e	e	e	e	e	e			
,, . .	ML	c	c	e	h	e	c	c	c	2	SB	5, 6·2
July. . .	5 UML	c	c	c	h	c	a	c	c	3	TSB	7·5, 6·2, 6·2
August .	5 U	a	a	a	h	a	a	a	a	3	TSB	10, 7·5, 11·2
,, . . .	ML	a	a	a	h	a	a	c	c			
September	5 UML	a	a	a	h	a	a	a	a	3	TSB	10, 6·2, 8·7
October .	5 UML	c	c	c	h	c	c	c	c	3	TSB	13·7, 11·7, 8·7
November	5 U	a	a	a	h	a	a	a	a	3	TSB	8·1, 10, 5·6
,,	LM	a	a	a	h	a	a	c	c			
December	5 UML	e	e	c	h	e	e	c	c	3	TSB	13·7, 11·2, 10
1926												
January .	6 UML	a	a	a	g	a	—	a	a	3	TSB	10, 7·5, 6·2
February.	6 UML	c	a	a	g(i)	a	a	c	c	2	TB	9·3, 5
,,	6 UML	a	a	a	g(i)	a	a	c	a	1	S	5
March . .	6 UML	a(c)	a	a	g	a	a	c	a(c)	3	TSB	9·3, 5·6, 5
April . .	6 UM	a	a	a	g	a	a	c	a			
	L	a	a	a	g(i)	a	a	c	a	2	TS	12·5, 7·5
,, . .	6 UM	c	a	a	g	a	a	c	c			
	L	c	a	a	g(i)	a	a	c	c	1	B	6·2
May. . .	6 UM	c	c	c	g	c	a	c	c			
	L	c	c	c	g	c	c	c	c	3	TSB	5·6

T.S.B.=top, side, base of shrub.
(c) walls; (i) in part; cuticle g or i.

LEAVES OF HERBS

Dianthus caryophyllus. — The xylem and fibres are acid throughout. The other tissues vary mainly from a to c (pH 5·9 to pH 5·6). The more acid range e (pH 5·2 to 4·8) occurs in most tissues in December (1 leaf), January (bases of 3 leaves), February (middle and base of 3 leaves). This acid range also occurs occasionally in the upper epidermis and the phloem, but the

Table XVII
Ligustrum vulgare

Date	Indicators Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Fibres	Guard cells	Lower epidermis	No. of leaves Position on shrub	Size in cms.
1925											
June . .	5 UML	c	c	c	h	c	c′	c	c	3 TSB	1·8, 1·2, 2·
July. . .	5 UML	c	e	e	h	e	e	e	e	1 T	2·5
,, . . .	5 UML	c	c	c	h	c	c	c	c	2 SB	2·5
	5 UM	c	c	c	h	c	c	c	c	3 TSB	1·8, 2·5, 3·
,, . . .	L	e	c	c	h	c	c	c	c		
September	5 UM	e	e	e	h	e	e	e	e	2 TB	2·5
,,	L	c	c	a	h	e	a	a	a		
	5 U	e	e	e	h	e	e	e	e		
	M	c	c	c	h	c	c	c	c	1 S	2·5
	L	c	c	a	h	c	a	a	a		
October .	5 UML	e	e	c	h	e	e	e	e	3 TSB	1·8, 2·5, 3·1
November	5 UM	e	c	c	h	e	e	e	e		
	L	e	c	c	h	e	e	e	e	3 TSB	3·7
December	5 UML	e	e	e	h	c	—	e	c	2 TS	3·7
,,	5 UML	e	e	e	h	e	—	e	e	1 B	3·7
1926											
January .	6 UML	a	a	a	g	a	—	a	a	1 T	3·7
,, .	6 UML	e	e	c	g	e	—	e	e	2 SB	4·3
February	6 UML	e	e	a	g	e	a	e	e	3 TSB	3·1, 3·1, 2·5
March . .	6 UML	e	e	e	g	e	—	e	e	1 T	2·5
,, . . .	6 UML	e	e	e	g	e	e	e	e	2 SB	3·1
April . .	6 UM	e	e	e	g	e	e	e	e		
	L	a	a	a	g	a	a	a	a	1 T	2·5
,, . .	6 UML	e	e	e	g	e	e	e	e	1 S	3·7
,, . .	6 UM	e	e	e	g	e	e	e	e	1 B	2·5
	L	c	c	c	g	c	c	c	c		
May . .	6 UML	e	e	c	g	c	e	e	e	3 TSB	2·5

T.S.B. — top, side, base of shrub.
c′ thin-walled fibres.

mesophyll, i. e. palisade and spongy parenchyma is acid only in the one December leaf and in the basal parts of the January leaves.

Table XVIII

Rhododendron ponticum

Date	Indicators Part	Upper epidermis	Hypodermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Fibres	Bundle sheath	Guard cells Lower epidermis	No. of leaves Position on shrub	Size in cms.
1925												
June . .	5 UM	e	e	e	e	h	e	e	e	e	3 TSB	4·3
	L	e	e	e	e	h	e	h	e	e		
July. . .	5 U	h	h	e	e	h	e	e	e	e		
	M	h	h	e	e	h	h	h	e	e	3 TSB	6·2
	L	h	h	h	h	h	h	h	h	h		
August .	5 UML	h	h	e	e	h	h	hk	e	e	3 TSB	7·5, 8·7
September	5 UML	h	h	e	e	h	h	h	e	e	3 TSB	10, 7·5
October .	5 UML	h	h	e	e	h	h	hk	e	e	3 TSB	10, 10, 6·2
November	5 U	e	e	e	e	h	h	e	e	e		
	ML	h	h	e	e	h	h	hk	e	e	3 TSB	5, 7·5, 5
December	5 U	e	e	e	e	h	h	hk	e	e		
	ML	e	e	e	e	k	h	hk	e	e	3 TSB	6·2
1926												
January	6 U	g	g	e	e	g	g	g	e	e		
	M	gi	gi	e	e	g	g	ik	e	e	3 TSB	8·1
	L	gi	gi	e	e	ik	g	ik	e	e		
February	6 U	g	g	e	e	g	g	g	e	e		
	M	gi	gi	e	e	g	g	g	e	e	3 TSB	7·5
	L	gi	gi	e	e	i	g	ik	e	e		
March . .	6 U	g	g	e	e	g	g	g	e	e		
	ML	gi	gi	e	e	i	g	i	e	e	3 TSB	6·2
April . .	6 U	g	g	e	e	g	g	g	e	e		
	ML	gi	gi	e	e	ik	g	ik	e	e	3 TSB	6·2
May . .	6 U	e	e	e	e	g	g	g	e	e		
	ML	e	e	e	e	i	g	ik	e	e	3 TSB	5·6

cuticle k.

Saxifraga umbrosa. — All the tissues throughout this leaf and throughout the seasons are in the range pH 4·4—4·0 (g h i), with one variation to pH 5·2—4·8 in May in the bundle sheath, lower epidermis and guard cells.

Limnanthes Douglasii. — This leaf is thin and very green, so that it was possible to investigate only the bases of the leaflets

Table XIX

Veronica andersoni × *V. sp.*

Date	Indicators Part	Upper epidermis	Hypodermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Bundle sheath	Guard cells and Lower epidermis	No. of leaves Position on shrub	Size in cms.
1925											
June . .	5 UM	e	e	e	c	h	e	c	e	3 TSB	2·5
	L	e	e	e	c	h	c	e	e		
July. . .	5 U	e	e	e	e	h	e	e	e	3 TSB	1·9
	ML	c	c	e	c	h	e	c	e		
August .	5 U	e	e	e	e	h	e	e	e	2 TS	2·5
	ML	e	c	e	c	h	e	e	e		
,, . .	5 UML	e	e	e	e	h	e	e	e	1 B	2·5
September	5 UM	e	e	e	e	h	e	e	e	1 T	2·5
	L	e	e	c	c	h	e	e	e		
,,	5 UM	e	e	e	e	h	e	e	e	2 SB	2·5
	L	e	e	c	a	h	e	e	e		
October .	5 UML	e	e	e	c	h	e	e	e	3 TSB	2·5, 1·9, 1·9
November	5 UM	e	e	e	e	h	e	e	e		
	L	e	e	c	a	h	e	e	e	3 TSB	2·5
December	5 UML	e	e	e	e	h	e	e	e	3 TSB	3·1
1926											
January .	6 UML	e	e	e	e	g	e	e	e	3 TSB	2·5
February	6 UML	e	e	e	e	g	e	e	e	3 TSB	2·5 2·5, 1·9
March . .	6 UML	e	e	e	e	g	e	e	e	2 TB	1·9
,, . .	UML	e	e	e	e	g	e	e	e	1 S	1·9
April . .	6 UML	e	e	e	e	g	e	e	e	1 T	1·9
,, . .	6 UML	e	e	e	e	g	e	e	e	1 S	1·9
,, . .	6 UM	e	e	e	e	g	e	e	e	1 B	1·9
	L	e	e	e	e	g	e	e	e		
May	6 UL	e	e	e	e	g	e	e	e	2 TB	3·7
	M	e	e	e	e		e	e	e		
,, . .	6 UML	e	e	e	e	g	e	e	e	1 S	2·5

Hairs e throughout where they occurred.

and the petioles. The letters UML are here (Table XIV) used to indicate the position of the leaflet on the leaf, *not* the upper, middle and basal portions of the leaflets. In the bases of the leaflets the pH values for all tissues except the xylem were mainly

Table XX. *Viburnum tinus*

Date	Indicators Part	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Xylem	Phloem	Fibres	Guard cells	Lower epidermis	Hairs	No of leaves Position on shrub	Size in cms
1925												
June . .	5 UML	e	e	e	h	e	e	e	e	he	2 TS	8·1, 7·5
	5 UM	e	e	e	h	e	e	e	e	he		
	L	e	e	c	h	h	h	e	e	he	1 B	6·2
July. . .	5 UML	e	e	e	h	e	e	e	e	hk	3 TSB	5, 3·7
August .	5 UM	e	e	c	h	e	—	e	e			
	L	e	e	e	h	e	hk	e	e	hk	3 TSB	6·2, 7·5
September	5 UML	e	e	e	h	e	e	e	e	h	1 T	6·2
„	5 UML	e	e	a	h	e	e	e	e	h	2 SB	7·5, 6·2
October .	5 UML	e	e	c	h	e	e	e	e	e	1 T	5
„ .	5 UML	e	e	c	h	e	h	e	e	e	1 B	3·3
„ .	5 U	e	e	c	h	e	h	e	e	e		
	ML	e	e	e	h	e	h	e	e	e	1 S	5
November	5 UML	e	e	e	h	e	h	e	e	e	3 TSB	4·2
December	5 U	e	e	a	h	e	h	e	e	e	3 TSB	7·5
	ML	e	e	e	hk	e	hk	e	e	e		
1926												
January .	6 U	e	e	e	g	e	—	e	e	e	3 TSB	6·2
	ML	e	e	e	gi	e	gi	e	e	e		
February	6 U	e	e	e	g	e	e	e	e	e	3 TSB	5
	ML	e	e	e	gi	e	ik	e	e	e		
March . .	6 U	e	e	e	g	g	e	e	e	e	3 TSB	5
	ML	e	e	e	i	g	i	e	e	e		
April . .	6 U	e	e	a	g	g	g	e	e	i		
	M	e	e	a	gi	g	i	e	e	i	1 T	5
	L	e	e	c	g	g	i	e	e	i		
„ . .	6 U	e	e	a	g	g	g	e	e	i		
	M	e	e	a	gi	g	i	e	e	i	1 S	5
„ . .	L	e	e	c	g	g	i	e	e	i		
	6 U	e	e	c	g	e	g	e	e	i		
	M	e	e	c	gi	g	i	e	e	i	1 B	5
	L	e	e	e	gi	g	i	e	e	i		
May . .	6 U	e	e	e	g	e	e	e	e	g		
	M	e	e	c	g	g	g	e	h	g	1 T	7·5
	L	e	e	e	i	g	i	e	h	ge		
	6 U	e	e	e(c)	g	e	e	e	e	g		
	M	e	e	e(c)	g	e	e	e	e	g	2 SB	6·2
	L	e	e	c	g(i)	e	e(i)	e	e	g		

of pH 5·9, with the lower value pH 5·6 appearing for all tissues in November and again in May, also in the epidermal tissues only for April.

More variation is shown by some tissues of the petioles. The usual reaction is pH 5·9, with pH 5·6 appearing in all tissues in one August petiole, in the epidermis for September, November, December, February, March, May and for one leaf in April. The epidermis, therefore, shows a marked summer period of lesser acidity in June, July and August, with another appearance in January. The cortex shows some cells pH 5·6 in May, as well as all cells in one August petiole. The ground tissue of the petiolar stele shows the acid range, pH 5·2—4·8, occasionally — May (3), July (1), January (2), thus contrasting periods of lower pH to the periods of higher pH in the epidermis. This ground tissue also varies from pH 5·6 to pH 5·9 being less acid in the spring February—March, and again in summer July (2) and August—November.

Primula vulgaris. — This species shows great variation but the main features may be summarised by eliminating the xylem, which is always acid, the fibres which show lesser acidity only in March-May, and the phloem which shows a consistently lower pH range (< 5·2) from October to February or March with a higher pH in the leaf-tip from March to October. The other tissues show a lower pH in January and February, in July in the lower portions of all leaves, and in August for all portions of one leaf.

LEAVES OF SHRUBS

Aucuba japonica. — The parenchymatous tissues of this species varies from pH 5·9 to pH 5·6 irregularly with the lower range pH 5·2—4·8 appearing in two June leaves and all three leaves for December.

Ligustrum vulgare. — The variation in this species is rather erratic, in the range pH 5·9— pH 4·0; but with the exception of one July leaf and the upper epidermis of the leaf base in August there is a distinct summer period of consistently higher pH in the parenchymatous tissues from June to August. With the exception of the spongy parenchyma and of one leaf in January there is also a winter period of general lower pH in these tissues, extending from October to February.

Rhododendron ponticum. — All the tissues in this species are in the lower pH range (5·2—4·0 or < 3·4). The xylem is always 4.4, and so are the phloem and fibres except in June and at the tip of the leaf in July. With the exception of the leaf base in July (**h**), the palisade and spongy parenchyma, the bundle sheath, lower epidermis and guard cells are always e (5·2—4·8). The upper epidermis and hypodermis are usually more acid (pH 4·4—4·0) but reach the higher pH range e in May and June, also in December and for the leaf tip only in November.

Veronica andersoni × *V. sp.* — This species also is usually of a lower pH e (5·2—4·8), but shows more variation and reaches the higher value c (pH 5·6) for most tissues in March (1 leaf), May (2 leaves) and in October (3 leaves). The higher value pH 5·6 appears occasionally in the palisade parenchyma and more frequently in the spongy parenchyma which reaches pH 5·9 in the lower portion of the leaves for September and November. There is a marked winter period of almost consistently greater acidity in all tissues, extending from November to February or March (2 leaves).

Viburnum tinus. — The differentiation obtained in the leaves of this species was noteworthy in several directions. The upper epidermis was constant (pH 5·2—4·8); the palisade parenchyma varied to a higher pH (5·6) only in the apical portions of leaves for October (1) and April (2). The spongy parenchyma varied from e to **a** irregularly with two periods of more or less general higher values, September — October and April. The phloem was nearly always pH 5·2—4·8 from May (2) to February, being more acid pH 4·4—4·0 from March to May (1). The lower epidermis and guard cells were usually pH 5·2—4·8, but the guard cells in one April leaf were pH 5·6 with the lower epidermis at pH 5·2—4·8 and again in one May leaf (T, ML) they were at pH 5·2—4·8 when the lower epidermis was at pH 4·4—4·0. These are noteworthy in being the only occasions on which a differentiation between guard cells and lower epidermis has been found during this investigation.

These changes are summarised in Table XXI, where the months are arranged from September onwards because the tabulation showed a natural break in the change periods as a whole at that point.

Now it can be seen that *Dianthus caryophyllus* has a less acid leaf with a winter period of lower pH. *Saxifraga umbrosa* has a more acid leaf with a May period of higher pH. *Limnanthes douglasii* has a less acid leaf with a slight fall in pH for November and May, together with two periods (January and summer) in which the ground tissue is more acid than usual while the epidermis is less acid than usual. *Primula vulgaris* leaf has most tissues less acid with a marked period of greater acidity (Jan.—Febr.) and some lower pH values in July—August. The phloem here shows a clear winter period (Nov.—Feby.) of higher acidity which extends in the middle and for basal parts of the leaf over the whole year except May and September.

Aucuba japonica has a leaf of higher pH with a lower pH in December and June. *Ligustrum vulgare* has a more acid leaf with a clear summer period of lesser acidity and except in the spongy mesophyll, a clear winter period of higher acidity. *Rhododendron ponticum* has a leaf of high acidity with short winter and summer periods of lesser acidity in the upper epidermis. *Veronica* hybrid has a leaf with a clear winter period (Nov.—Mar.) of general higher acidity. *Viburnum tinus* leaf shows a spring (Mar.—May period of increased acidity in the phloem and also short autumn and spring periods of decreased acidity in the mesophyll.

The winter period (Nov.—Mar.) is thus a period of higher acidity in leaves and the changes to lower acidity occur at various times from April to October. The chief exception to this general rule occurs in the very acid upper epidermis of Rhododendron leaf which is less acid in November—December.

Comparing this with what happens in the stems investigated we find that there the only tissue internal to the sub-epidermis which shows a lesser acidity in winter is the pith of *Ligustrum* stem; and we find that for stems the epidermis (*Senecio*, *Aucuba*), and sometimes the sub-epidermis (*Lamium*), and the hairs (*Cerastium*, *Cheiranthus* shade, *Viburnum*) are the tissues which, like the Rhododendron leaf upper epidermis, are less acid in winter.

The occasional absence of critical differentiation between one tissue and another in these R.I.M. investigations of stems and leaves has already been noted (p. 59). The use of indicator solutions containing more than 10 % alcohol may have resulted in a mixing of saps from the killed cells, but the considerable

Table

Summary of

Plant	Tissues	Sept.	Oct.	Nov.	Dec.
Dianthus	Most				+ (1)
	Mesophyll				+ (1)
Saxifraga	B. Sheath				
	L. Epid. & G. Cells				
Limnanthes	Leaflet Bases				
	Most			+	
	Epidermis				
	Petioles				
	Most				
	Epidermis	+		+	+
	Cortex				
	Ground Tissue				
Primula	Fibres				
	Phloem	—	+ (ML)	+	+
	Most				
Aucuba	Most			+	
Ligustrum	Most				
	Except S. Paren		+	+	+
Rhododendron	Most				
	Upper Epid.			+ (U)	
	Phloem & Fibres				
Veronica	Most		—	+	+
Viburnum	Phloem				
	Palis. Paren.		— (1)		
	Spongy Paren.	— (2)	—		+ (U)

Notes: + = more acid; — = less acid. (1) (2) = one, two leaves only.

differentiation obtained in *Primula*, *Viburnum* leaf, and others indicates that this factor was not important with all materials; a conclusion which is supported by INGOLD's work on *Pelargonium* (p. 56). Further the chief cases of uniformity occurred in organs which were, like *Pelargonium* stem, of the "acid type". The possible mixing of saps is, therefore, to be regarded as a factor of possible importance in detailed differentiation, such as that of guard cells and lower epidermis, but as of minor importance when general seasonal variations are being considered.

XXI

leaf Changes

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
+ (L)	+ (ML)						
+ (L)							
				—			
				— (ML)			
				+			
			+				
							+ (1)
	+	+	+ (1)	+			+ (1)
				+			+ (1)
				(cells)			
+ (2)				+	+	+ (1)	
		—	—	—			
+	+	+ (ML)	+ (L)	—	+ (ML)	+ (ML)	+ (ML)
						+ (ML)	+ (1)
					+ (2)		
					—	— (2)	—
+ (2)	+						
						+ (L)	
				—	—		
					—	— (U)	
+	+	+ (2)		— (2)			
		+	+	+ (1)			
			— (2)				
			—				

(U) (M) (L) apical, middle, basal part of leaf.

Considering the different seasonal changes found in the various tissues, the lack of agreement between these results and the autumn period of decreased pH found by ABBOTT in the juice of apple and peach tips is not surprising. It is also possible that tissue investigations of the apple and peach material might confirm ABBOTT's data for the mixed juices.

With all the possible variables it is easy to theorise; there may be fluctuations in 1. an acid-producing metabolism, or 2. an acid-using metabolism, or 3. a buffer-producing or buffer-using metabolism. As a speculation it might be suggested that the winter decreases of acidity in external cells take place in cells with an acid-producing metabolism which is governed by temperature and/or light conditions; while the summer periods of lesser acidity for inner tissues take place in cells where the increased efficiency of metabolism results in the utilisation of acids and/or an increase of the buffer index of the sap in the region of the natural pH.

CHAPTER XII

THE SUNFLOWER. (HELIANTHUS ANNUUS)

TISSUE REACTIONS AND BUFFER SYSTEM

As one example of a particular species investigated, from the present point of view, in some detail we take the Sunflower (*Helianthus annuus*). This investigation was carried out, as part of our survey, by Miss S. H. MARTIN who published the results in three papers contributed during the years 1926—1927 to *Protoplasma* Vols. I and III.

1. THE TISSUE REACTIONS OF SUNFLOWER

These are given below as reported upon by Miss MARTIN with some small emendations and eliminations.

METHODS OF RESEARCH

Sunflower (*Helianthus annuus*) was studied in detail at all stages (as far as possible) in the life history of the plant from the seed to the mature flowering plant. The reactions throughout were obtained by noting the behaviour of the various tissues, towards certain indicators, as shown by sections.

The „range indicator method" as described by Professor SMALL (1926) was used. The sections were washed in neutral water before being placed in indicator solution. In most cases the staining was allowed to proceed overnight but, lest this should make a difference, sections in stain for a short time (usually about one hour) were compared with those left overnight. The latter sections were more deeply stained but gave exactly the same quality of colour, provided suitable precautions were taken to prevent any access of acid or alkali to the indicator solutions while staining was proceeding.

Neutral water was obtained by mixing the required amount of tap water which gave an alkaline reaction i. e. $pH > 7{\cdot}0$ with distilled water which was acid in reaction i. e. $pH < 7{\cdot}0$.

The indicator used for this purpose was P. R. Phenol sulphonphthalein which indicates, i. e. gives marked colour changes, between pH 6·6 yellow and pH 8 red.

As the point of neutrality is that of least colour in the solution, by adding tap water to the more acid distilled water till the solution of indicator and water becomes practically colourless the neutral point, pH 7 — not appreciably more or less, is obtained.

After staining, the sections were again washed in neutral water and examined in daylight with the low power of the microscope.

With few exceptions all the reactions of the tissues came within the useful range of five indicators. These were BPB, BAN, MR, DER and BCP.

Where these indicators did not cover the range, as in the case of some of the epidermal hairs and the callus on the sieve plates in the phloem, other suitable indicators were used.

Alcoholic solutions of the dyes as prepared by the British Drug House were used.

Fruits were soaked in neutral water and their reactions studied. Some seeds were germinated on blotting paper while others were germinated in soil. Some difficulty arose in obtaining suitable stages of seedlings. At first these were compared at different ages but as the plants grew so slowly and the rate of growth was so irregular, due to weather conditions &c., it was found necessary to take stages of development as convenient points from which results could be recorded and the different parts of young growing plants could be compared. For example plants with one pair of leaves were compared with those having two pairs, and so on to maturity.

At all stages sections were examined at various levels such as stem close to soil level, stem high up (i. e. close to the apex) and at intermediate levels, petiole of leaf near the stem, close to lamina, leaf at tip and at base and so on with all parts of the plant. At least three plants at each stage were examined, and in every case a control unstained section, was compared with the stained one so as to avoid any confusion of natural colouration, such as that due to chlorophyll etc., with indicator effect.

In cotyledons and leaves it was sometimes difficult to observe the reaction of the cell-contents owing to the dense green colour of the chloroplasts.

An attempt was made to study the reactions of the guard cells of the stomata, but as these cells had rather dense contents and also contained chloroplasts all records of such reactions must be doubtful. So far as could be observed the acidity of these guard cells did not differ from that of the neighbouring epidermal cells.

The R. I. M. has the advantage of facility in working, and by the use of several indicators with overlapping pH ranges gives accurate results with a better view of the reactions of the separate tissues than any determinations of the hydrion concentration of expressed sap.

An objection to the washing of tissues in water might be raised as this might dilute the cell sap thus altering its reaction; but it has been found that expressed sap may be diluted to twice its volume without any marked change in reaction, and further the reaction of the cell contents is in most cases determined in unbroken cells, therefore the effect of such washing will be negligible.

Injury effects are possible but so far no method has been devised by which the actual acidity of plant cells in the living plant may be measured. This acidity of the normal plant may vary considerably from that of the plant injured by cutting, but control of the injury factor is as yet impossible (see p. 266).

Purple stemmed seedlings were compared with the ordinary green stemmed variety used throughout this work but no differences were detected. The purple colour of the epidermal cell contents obscured the reaction of these.

RESULTS

In the Tables given below the symbols for the pH ranges, as explained on p. 49, are used. Commonly occurring ranges are 5·9—5·6 = b, 5·6 = c, 5·6—4·8 = d, 5·2—4·8 = e, 5·2—4·4 = f, 4·4—4·0 = h, 4·0 = i, < 3·4 = k. Symbols in brackets indicate ranges which were seldom observed and which are not considered to be the normal reactions.

Tissue Reactions of Helianthus annuus

by S. H. Martin

Table I (Seed)

Seed	Plumule	Mesophyll of Cotyledons	Epidermis	Sub.-Epid.	Vascular Strands	Cap	Tip	Plerome	Procamb. Strands	Inner Periblem	Outer 2—3 Layers Periblem	Dermatogen
		Cotyledons				Root						
1. Ungerm. . . .	e	e	h	h	h	h	h	c	h	e	h	h
2. Germ. in soil .	e	e	h	h	h	h	h	e	h	c	h	h
3. Germ. on B. P.	e	e	h	h	h	h	h	e	h	c	h	h

Table II (Cotyledons)

Stages	A Seed Ungerm.	B Seed Germ.	C Cots. Expd.	D 1st Fol. Leaf.	E 2nd Fol.	F 3rd Fol.	G 4th Fol.	H 5th Fol.
Epidermis . . .	h	h	h	h	h (k)	h	h	h
Sub-Epidermis .	he	h	eb	e	hb	e	e	e
Palisade	e	e	eb	e	eb	e	e	e
Spongy Mesophyll	e	e	eb	e	eb	e	e	e
	unlign.							
Xylem	e	e	h	h	h	h	h	h
Phloem	e	e	e	e	e	e	e	e

Table III (Petiole of Cotyledon)

Stages as before	A	B	C	D	E	F	G	H
U. Epidermis.	—	—	h	h	h	h	h	h
Sub-Epidermis	—	—	h	h	h	h	h	h
Cortex . . .	—	—	e	b	eb	eb	b	b
Phloem . . .	—	—	e	e	eb	e	e	e
Xylem . . .	—	—	h	h	h	h	h	h
L. Epidermis	—	—	h	he	he	e	he	e

Table IV (Stem-upper)

Stages	Epidermis	Epidermal Hairs	Sub-Epidermis	Outer Cortex (collenchyma)	Inner Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Pith
		and										
I Cots. Expd. .	h	e (10—9)	he	b	b	b (e)	b (e)	—	be	- - -	h	b
II 1st Fol.	he	e (10—9)	eb	eb	be	be	be	- -	e	be	h	be
III 2nd ,, ...	he	e (10—9)	e	e	be	be	be	—	e	e	h	be
IV 3rd ,, ...	he	e (10—9)	h	b	b	b	b	—	eb	be	h	b
V 4th ,, ...	h	e (10—9)	e	e	b	b	b	—	e	e	h	b
VI 5th ,, ...	h	e (10—9)	e	e	e	e	e	—	e	e	h	e
VII 6th ,, ...	h	e (10—9)	ba	ba	ba	ba	ba	—	ba	ba	h	ba
VIII Flrng. Indoor .	he	e (10—9)	e	b	b	b	b	—	be	be	h	b
IX Flrng. Outdoor	h	e (10—9)	e	be	be	be	be	—	be	be	h	be

Table V (Stem—middle)

Stages	Epidermis	Epidermal Hairs	Sub-Epidermis	Outer Cortex (collenchyma)	Inner Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Pith
I Cots. Expd. .	h	and e (10—9)	h	be	b	b	b	—	e	e	h	b
II 1st Fol.	h	e (10—9)	e	e	b	b	b	—	e	e	h	b
III 2nd „	h (e)	e (10—9)	e	be	b	b	b	—	e	e	h	b
IV 3rd „	h	e (10—9)	he	be	b	b	b	h	e	e	h	b
V 4th „	h	e (10—9)	e	b	b	b	b	h	e	e	h	b
VI 5th „	h	e (10—9)	e	b	b	b	b	h	b	b	h	b
VII 6th „	h	e (10—9)	ea	a	a	a	a	h	ba	ba	h	b
VIII Flrng. Indoor .	h	e (10—9)	eb	b	b	b	b	hk	be	be	h	b
IX Flrng. Outdoor	h	e (10—9)	h	b	b	b	b	k	b	b	h	b

Sieve plates 6·2—5·9 or 6·2 ca.

Table VI (Stem—just above soil)

Stages	Epidermis	Epidermal Hairs	Sub-Epidermis	Outer Cortex (collenchyma)	Inner Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Pith
I Cots. Expd. .	h	—	he	b	b	b	b	—	be	—	h	b
II 1st Fol.	h	—	he	be	b	b (e)	b (e)	—	e	e	h	b
III 2nd „	h	—	he	be	b	b	b	—	e	e	h	b
IV 3rd „	h	—	he	e	be	b (e)	b (e)	h	be	be	h	b
V 4th „	h	—	he	e	b	b	h	h	e	e	h	b
VI 5th „	h	—	he	e	b	b	b	h	e	e	h	b
VII 6th „	h	—	h	a	a	a	a	h	b	b	h	a
VIII Flrng. Indoor .	h	—	e	be	b	b (e)	b	k	be	be	h	b
IX Flrng. Outdoor	h	—	h	b	b	b (e)	b (e)	k	e	e	h	b

Table VII (Stem—just below soil)

Stages	Epidermis	Epidermal Hairs	Sub-Epidermis	Outer Cortex (collenchyma)	Inner Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Pith
I Cots. Expd. .	h	—	—	h	e	he(k)	e	—	e	e	h	e
II 1st Fol. . . .	k	—	—	hk	e (b)	he	he(b)	—	e	e	h	e (b)
III 2nd „ . . .	hk	—	—	hk	e (b)	h	he	h	e (b)	e	h	e (b)
IV 3rd „ . . .	k	—	—	h	e	h	e	h	e	e	h	e
V 4th „ . . .	hk	—	—	h	e	h	he	h	e	e	h	e
VI 5th „ . . .	hk	—	—	h	e	h	h	h	e	e	h	e
VII 6th „ . . .	k	—	—	h	eb	h	e	h	e	e	h	e (b)
VIII Flrng. Indoor	hk	—	—	e	e (b)	he	he	hk	e (b)	e	hk	e (b)
IX Flrng. Outdoor	hk	—	—	ek	e	h	h	k	e	e	hk	e

Sieve plates 6·2—5·9 or 6·2 ca.

Table VIII (Root—upper level of 2ndy roots)

Stages	Piliferous Layer	Exodermis	Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Xylem Fibres	Pith
I Cots. Expd. . .	h	h	e	hke	h	—	e(b)	-	h	-	e
II 1st Fol.	hk	he	e	hk	hk	—	e	e	h		e
III 2nd „	hk	he	e	hk(e)	hk	—	e	e	h	h	e
IV 3rd „	hk	h	e	h	he	h	e	e	h	h	e
V 4th „	k	h	e	h	he	h	e	e	h	h	e
VI 5th „	k	h	e	hk	h	h	e	e	h	h	e
VII 6th „	k	h	e	h	e	h	e	e	h	h	e
VIII Flrng. Indoor .	hke[1]	e	e	h	he	k	eb	eb	h	hk	e
IX Flrng. Outdoor .	hke[1]	hk	e[2]	h	h	k	e	e	h	k	e

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1) Inner wall e.

2) Few cells = k.

Table IX (Root—middle high up)

Stages	Piliferous Layer	Exodermis	Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Xylem Fibres	Pith
I Cot^s^. Expd. .	h	h	e	he(k)	e	—	e	e	h	—	e
II 1st Fol. . . .	h (k)	he	e	he(k)	e(k)	—	e(b)	e	h	—	e
III 2nd ,, . . .	hk	he	e	he(k)	e(k)	—	e	e	h	—	e(b)
IV 3rd ,, . . .	k	h	e	h	he	—	e	e	h	—	e
V 4th ,, . . .	k	h	e	h	he	—	e	e	h	—	e
VI 5th ,, . . .	k	h	e	k	h	—	e	e	h	—	e
VII 6th ,, . . .	k	h	b	h	e	—	e	e	h	—	b
VIII Flrng. Indoor .	h	e	e	h	he	—	eb	e	h	—	e
IX Flrng. Outdoor	—	e	ek	h	h	—	e	e	h	—	e

Sieve plates 6·2—5·9 or 6·2 ca.

Table X (Root—near tip)

Stages	Piliferous Layer	Exodermis	Cortex	Endodermis	Pericycle	Pericyclic Fibres	Phloem	Cambium	Xylem	Xylem Fibres	Pith
I Cots. Expd. .	h	he	e	h	he	–	e	e	h	--	
II 1st Fol. . . .	k	e	e	h	e	—	e	e	h	—	--
III 2nd ,, . . .	hk	e	e	h	he	—	e	e	h	—	—
IV 3rd ,, . . .	k	he	e	h	e	—	e	e	h	—	—
V 4th ,, . . .	k	e	e	h	he	—	e	e	h	—	—
VI 5th ,, . . .	k	e	e	h	h	—	e	e	h	—	—
VII 6th ,, . . .	k	b	b	h	e	—	e	e	h	—	—
VIII Flrng. Indoor .	h	be	be	h	e	—	e	e	h	—	—
IX Flrng. Outdoor	k	be	e	h	h	—	e	e	h	--	--

Table XI (Leaf)

Stages	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Phloem	Xylem	Lower epidermis	Hairs epidermal
							and
I Cots. Expd. .	h	e	e	e	h	h	e (10 9)
II 1st Fol. . . .	h	e	e	e	h	h	e (10--9)
III 2nd ,, . . .	h	eb	b	e	h	h	e (10--9)
IV 3rd ,, . . .	h	eb	eb	e	h	e	e (10—9)
V 4th ,, . . .	h (e)	e	e	e	h	h	e (10—9)
VI 5th ,, . . .	e	e	e	e	h	e	e (10—9)
VII 6th ,, . . .	h	e	a	a	h	a	e (10 - 9)
VIII Flrgn. Indoor .	h	b	b	b	h	b	e (10- 9)
IX Flrng. Outdoor	h	b	b	b	h	b	e (10---9)

Sieve plates 6·2—5·9 or 6·2 ca.

Table XII (Petiole of Leaf)

Stages	Upper epidermis	Sub-Epidermis	Cortex	Phloem	Xylem	Lowre epidermis	Hairs
I Cots. Expd. .	—	—	—	—	—	—	
II 1st Fol. . . .	—	—	—	—	—	—	and
III 2nd ,, . . .	h (b)	eb	b	eb	h	h (b)	e (10--9)
IV 3rd ,, . . .	h	e	e	e	h	h	e (10—9)
V 4th ,, . . .	h	h	b	e	h	he	e (10—9)
VI 5th ,, . . .	h	e	b	e	h	he	e (10—9)
VII 6th ,, . . .	h	a	a	a	h	h (b)	e (10—9)
VIII Flrng. Indoor .	h	e	b	e	h	h	e (10—9)
IX Flrng. Outdoor	h	h	b	e	h	h	e (10—9)

Sieve plates 6·2—5·9 or 6·2 ca.

Table XIII (Flower heads.) At all stages examined

Gynoecium			Androecium
Ovary Wall			
Pericarp	e		
VBS. in Pericarp	h		
Ovule	e		
Style (Young)	e	e	Stamens (Young)
„ (Mature)	h or k	b	Anthers (Mature)
Meristem at Top of Ovary	h-k	e	Filaments
		e	Pollen-Grains

Corolla		Receptacular Scales	
Epidermis	hk	VBS. Xylem	h
Inner Tissue	e	Phloem	e
		Ground Tissue	ek

Top of Receptacle	e and k (patches)
Base „ „	ee

Involucral Bracts

Epidermis (Upper)	h
Palisade parenchyma	he
Spongy „	e
Xylem	h
Phloem	e
Epidermis (Lower)	he
Hairs	eb

DISCUSSION OF RESULTS

The results tabulated above show how by means of suitable indicators it is possible to map out the distribution of relatively acid and relatively alkaline tissues in sections of the various regions of any plant. For example, we find in a transverse section of sunflower stem that the contents of the epidermal cells, the walls of the pericyclic fibres, and the walls of the xylem vessels and fibres are more acid than the other tissues of the stem.

TISSUES

Epidermis and epidermal hairs. If sections of ungerminated seeds are taken it is found that the epidermis is relatively acid from the first. The dermatogen of the radicle gives the reaction pH 4·4—4·0. The epidermis of the cotyledon agrees with this; and this acid epidermis persists right up through the various seedling stages to the mature plant where it is found in stem and leaf and even in the corolla of the young flower.

In a few cases, especially in the hypocotyl below soil of the mature plant the epidermis has an acidity as high as pH $<$ 3·4. In some cases where this abnormally high acidity of the epidermis occurs we find some cells of the cortex which appear to be injured and which also show the very acid reaction of the uninjured epidermis.

The epidermal hairs do not correspond in acidity with the cells of the epidermis itself but show interesting differences, all the hairs being more alkaline and some being the most alkaline part of the plant. The hairs on leaf and stem vary from pH 4·8—5·2 to a pH $>$ 9 being alkaline to B. P. B., B. T. B., P. R. N. R., C. R. and T. B., and acid to Phenolphthalein. The type of hair one cell thick from the base upwards was more acid, (being at pH 4·8—5·2) than the hair with the multicellular base which gave a deep blue colour with T.B. and was colourless with Phenolphthalein, indicating a pH $>$ 9 and $<$ 10. The multicellular base of the hair was less alkaline than the tip in some cases. These more alkaline epidermal hairs were the only cells which showed a reaction on the alkaline side of neutrality.

Piliferous layer. The piliferous layer and the root hairs were acid at all stages. The reaction given by this layer was pH 4·4—4·0 sometimes pH $<$ 3·4, and as in the case of the epidermis of the hypocotyl underground the higher acidity i. e. pH $<$ 3·4 may have been due to injury of the cells.

Exodermis. In young seedlings before the first foliage leaves were expanded the exodermis was more acid than the cortex and corresponded in acidity with the piliferous layer, i. e. pH 4·4—4·0. In older seedlings the reaction of the exodermis was the same as that of the cortex i. e. pH 5·2—4·8. Where crushed cells or cells injured in some way were found in the exodermis these gave the higher reaction i. e. pH 4·4—4·0, and sometimes pH $<$ 3·4.

Cortex. The records of results given for the outer cortex in the stem regions are rather conflicting. In some cases the outer cortex gives an acidity as high as or almost as high as that of the epidermis; in other cases this is not so and the pH of the outer cortical cells corresponds to the pH value of the inner cortex. This confusion may be due to the rather dense contents of the collenchymatous cells and possibly also to the reaction of the walls. The reaction of the walls of ordinary thin walled parenchymatous cells was not studied as cellulose seems to take up the indicators used so very slightly that no positive results could be recorded. In the case of collenchyma also the colours were very faint but the reaction of the walls seemed to be as follows; — Blue with B. P. B., Pink (pale red) with B. A. N., Pink with M. R., Pink with D. E. R and Yellow with B. C. P. indicating a pH of 4·4—4·0, but the staining was so very slight that records must remain doubtful.

The inner cortical cells of the stem vary from pH 5·9—5·6 to pH 5·9 ca. the reaction with few exceptions being the former. In a very few cases the inner cortical cells were as acid as pH 5·2—4·8.

In the regions of the stem close to soil level above ground and just below soil level the variations found in each are such as would be expected, because the portions just below ground from which sections were taken may not have been equally ‚earthed' and the CO_2 consequently may not have accumulated to the same extent, and the regions just below soil level in some cases may have been under conditions similar to those parts just above soil level, so that the occurrence of the same reactions in the cortical parenchyma of each, in some cases at least, may be due to the effect of the accumulation of the CO_2 round these parts.

The usual reaction given by the hypocotyl a short distance above soil level was pH 5·9—5·6 with pH 5·2—4·8 as the exception while just below soil the reaction pH 5·2—4·8 occurred most frequently and pH 5·9—5·6 was the exception.

The cortical cells of the root and of the hypocotyl below soil are on the whole slightly more acid than those of aerial parts of the plant. This difference is due, no doubt, to the penetration into the cells of soil CO_2 or to the non-escape of the CO_2 of respiration, or both factors may operate (cp. CERIGHELLI 1920 and MAGNESS 1920).

In this respect it is interesting to compare seedlings brought on entirely in the dark in a closed cupboard and young sunflower plants kept in the dark for a week or so only, with normal i. e. non-etiolated seedlings.

The parenchymatous cells of the former were slightly more acid than those of the latter, thus agreeing more nearly with the corresponding tissues in roots and underground parts.

It was some time before the differences were detected as thin sections of each gave practically no colouration with M. R. and it was only on taking quite thick sections that a pink colour was seen in the cortex and pith of the hypocotyl of etiolated seedlings (a pink colour denotes an acid reaction towards M. R.).

The reaction given by the cortical cells in the roots and underground hypocotyl regions was pH 5·2—4·8 with a pH of 5·9—5·6 in one plant only.

Endodermis. In some roots and sometimes in the hypocotyl underground an acidity as high as pH $<$ 3·4 was found but usually the endodermis gave the reaction pH 4·4—4·0, sometimes as low as pH 5·2—4·8 which was the usual figure for the cortex and pith in subterranean parts.

This difference in acidity of a tissue below ground as compared with that of the same tissue found in aerial parts of the plant was most marked in the case of the endodermis and the pith, but occurred also in other tissues (see Tables VI—VII).

The endodermis of the aerial parts of the plant, in all cases examined, showed no difference in acidity from that of the neighbouring cortical cells, and varied from pH 5·9—5·6 to pH 5·9 ca. and was seldom at pH 5·2—4·8. Where the cortical cells were at pH 5·2—4·8 the endodermis also gave this reaction.

Pericycle. As in the case of the endodermis the acidity of the parenchymatous cells of the pericycle in underground parts was sometimes as high as pH $<$ 3·4, but more often the reaction was pH 5·2—4·8 thus corresponding in acidity with the parenchymatous cells of the cortex and pith in underground regions.

pH 4·4—4·0 was also found in a number of cases in the root and hypocotyl below soil.

Above ground the reaction of the pericycle was the same as that of the cortex and pith i.e. pH 5·9—5·6 or in some cases pH 5·9 ca.

The walls of the pericyclic fibres varied from pH 4·4—4·0 to an acidity as great as or more than pH 3·4, this being the limit on the acid side of the range of indicators in use.

If sections of the stem of mature sunflower are taken at different levels from the stem apex it will be seen that the region of the pericyclic fibres as yet unlignified is less acid (being blue with B. P. B.) than the corresponding region a short distance down the stem where the pericyclic fibres now slightly lignified show a green colour with B. P. B. Still further down where lignification is complete a yellow colour is produced with this indicator showing that the fully lignified pericyclic fibre is one of the most, if not the most, acid element of the mature Sunflower stem.

Phloem. In most cases in all parts of the plant the phloem elements were found to be at pH 5·2—4·8 possibly slightly more alkaline in a few cases.

The phloem is sometimes much more alkaline than the xylem having occasionally an acidity as low as pH 5·9 ca. and, where callus is developed, the sieve plate gives a reaction as low as pH $>$ 6·2 being alkaline to B. C. P. i. e. giving a blue colour with this indicator. With B. T. B., however, no trace of blue was observed so that the callus is acid to B. T. B. The hydrion concentration therefore lies between pH 6·2—5·9, or possibly pH 6·2 ca.

The contents of the sieve-tubes, companion cells and phloem parenchyma gave the same reaction, i. e. pH 5·2—4·8 usually.

In mature sunflower grown out of doors the phloem sometimes gives a reaction as low as pH 5·9 ca. Where callus was developed, using the indicator B. C. P. the sieve plates showed up very clearly as deep blue plates against the decided yellow of the sieve-tube contents. As in the case of lignification, though in the opposite direction, the chemical nature of callus may be responsible for the more alkaline reaction. Apart from the callus, in sunflower the phloem is usually slightly more acid than the cells of the cortex and pith. No differences were traced between the acidity of the phloem in parts above and below soil level though other regions of the stem e. g. cortex, pith, endodermis and pericycle were more acid below ground than above.

Cambium. Actively dividing fascicular and interfascicular cambial cells gave the ordinary reaction of the neighbouring cortical cells though the staining was rather more intense owing to the denser contents of the cells. Here the nuclei were deeply stained but the colour was of the same kind.

In the mature sunflower phellogen was found in the outer cortex of the stem. The reactions given by these cells showed that the outer cells cut off by the phellogen cells were more alkaline than the inner cells i. e. the phellogen cells; the reactions given by these were pH 5·2—4·8 for the outer cells as compared with pH 4·4—4·0 for the inner cells. With B. A. N. these outer cells were a clear orange (pink with M. R.), while shrivelled up dead cells on the outside were quite purple red.

It would have been interesting to study corky tissue in fully suberised cells or during the process of suberisation in sunflower, but these were not available.

Elder cork was examined. Here the cambium was at pH 5·2—4·8 while the suberised tissue gave a reaction of pH $<$ 3·4. According to PEARSALL and PRIESTLEY (1923) this higher acidity is due to the liberation of fatty acids during suberisation.

Xylem. The xylem elements vary from pH 4·4—4·0 to an acidity as great as or more than pH 3·4. This relatively high acidity is especially marked in the xylem fibres; the vessels do not seem to be so acid but vary from blue to green with B. P. B., reddish orange to red with B. A. N., red with M. R. and D. E. R. and yellow with B.C.P. giving a pH of 4·4—4·0 as compared with the pH $<$ 3·4 of fully lignified fibres. As in the case of the pericyclic fibres, by studying the reactions of the xylem elements at different distances from the stem apex the change in reaction during lignification may be observed.

This relatively high acidity of the mature xylem fibre, and of the mature pericyclic fibre, is due no doubt to the chemical change which takes place during the process of lignification, the impregnation with lignin of the original cellulosic walls causing these walls to give a more acid reaction.

Medullary rays. The parenchymatous cells of the medullary rays were at pH 4·4—4·0 sometimes and occasionally as relatively alkaline as pH 5·2—4·8 in underground parts, where they corresponded in acidity with the pith and cortex, and pH 5·9—5·6 in aerial parts. In underground parts of the mature sunflower especially in the region of the phloem the medullary rays were decidedly more acid being as high as pH $< 3{\cdot}4$. In these cases browning of the tissues was observed; the parenchymatous cells of the cortex also gave a very high relative acidity and showed browning on being exposed to air.

Pith. The central pith cells gave the same reactions as the cortical parenchyma, the usual reaction being pH 5·9—5·6 for aerial parts and pH 5·2—4·8 for subterranean parts.

The parenchymatous cells adjoining the xylem sometimes were more acid and gave a pH of 4·4—4·0.

COTYLEDONS AND FOLIAGE LEAVES

No differences in the reactions of leaves from various levels on the plant were detected.

It was found very difficult to study the actual pH of the assimilating tissue owing to the dense green colouration of the mesophyll but the conclusion arrived at was that in most cases the mesophyll gave the reaction pH 5·2—4·8 and that it was sometimes more alkaline i. e. pH 5·9—5·6.

GUSTAFSON (1924) found that the younger leaves of another sunflower (*H. multiflorus*) were more acid than the lower leaves.

The parenchymatous cells of the mid-rib of the leaf and also the xylem and phloem gave reactions similar to those of the corresponding tissues in the stem.

The tissues of the petioles of the cotyledons and leaves also agreed in acidity with the reactions of the same tissues of the stem.

FLOWER BUDS AND PRIMORDIA

It was interesting to find that even the very young developing flower buds arising as little protuberances on the top of the receptacle were much more alkaline than the more acid cells of the receptacle, being a deep blue with B. P. B. and yellow with B. A. N. indicating pH $> 4{\cdot}8$ as compared with pH $< 3{\cdot}4$ for the more acid cells of the receptacle. The upper region of the receptacle with B. P. B. gave bright patches of yellow cells. The very acid patches were probably crushed cells where the contents were exposed to oxidation by an oxidase present in the cells.

The bracts of the flowers were sometimes yellow, sometimes blue and sometimes patchy yellow and blue with B. P. B. In older flower buds the meristematic region at the junction of the ovary with the other parts of the flower was also acid. The corolla when developing showed an acid epidermis but in the older flower buds reactions were masked by natural colour. Pollen grains showed the same acidity, i. e. pH 5·2—4·8, as the young developing flower buds. The styles as far as could be made out were more acid giving a deep red with B. A. N. indicating pH 4·4—4·0 or possibly $<$ 3·4 for the mature style. This reaction again was obscured by natural colouration the mature style being deep yellow with some yellow and some purple papillae.

The ovary and the ovule at all stages gave the same reaction as all the parts of the young developing flower buds i. e. pH 5·2—4·8. In the mature flower the conducting strands in the wall of the ovary showed a pH of 4·4—4·0.

As developing flower buds respire more actively than any other part of the plant it may be that some at least of the relatively high acidity is due to the CO_2 accumulated in the unopened buds but if this is the case one wonders why the basal region of the buds still remains relatively alkaline while arising from a receptacle which is so much more acid.

Flower buds at all stages (as far as possible) were taken, and even in the very youngest where primordia were just beginning to develop this phenomenon was found. It seems more likely that oxidation of the tissues is the primary cause of the results observed especially as older opened heads and fully developed flower heads gave the same results and browning of these parts was actually observed.

Compositae are included in a list of Oxidase Families given by ONSLOW (1923) and marked darkening of exposed sap of *Helianthus multiflorus* on exposure to air is mentioned by ATKINS in Recent Researches (1916).

Increased acidity and pigmentation due to oxidation of tissues

That a greater acidity due to the oxidation of tissues does occur has been recorded by MCCLENDON and SHARP (1919). These workers record a change in expressed sap of carrots from pH 5·85 to pH 5·73 on standing for twenty minutes in air. HAAS (1920) observed a change of reaction from pH 6·12 to pH 5·91 in the juice of clover plants left overnight.

Browning of tissues was observed in roots and in the underground hypocotyl region of the mature sunflower and in sunflower head.

In these regions we should expect to find accumulation of certain metabolic products, i. e. sugars, which according to ONSLOW are capable of taking part in the reactions involved in pigment formation. OVERTON (1899) was the first to observe that excess sugar might cause the formation of pigments. He experimented with a number of plants, feeding them with sugar, and found that in many species of Monocotyledons and Dicotyledons, both water and land plants, sugar feeding will bring about anthocyanin formation. The question of the results of sugar feeding has been taken up by other writers, including ONSLOW who assumes that the chromogen is formed from sugars in the leaf and that increase in amount of sugar leads to increased formation of chromogen with the resultant production of anthocyanin unless the chromogen be removed. The chromogen flavone may be removed under certain circumstances by combination with sugar to form a glucoside and water, this reaction involving the substitution of sugar molecules for hydroxyl groups of the flavone.

The general reactions given by ONSLOW (1916) are:

1. Glucoside + water $\rightleftarrows$ chromogen + sugar.
2. x (chromogen) + oxygen $\longrightarrow$ anthocyanin.

A glucosidase, i. e. a glucoside—hydrolysing enzyme or enzymes, probably catalyses the first reaction and an oxidising enzyme controls the second.

This second reaction may be initiated or accelerated by a relatively acid medium, such as that observed, or both reactions may be affected. On hydrolysis of the flavones, present as glucosides, with dilute acids the sugar is split off and the colour formation by oxidation may then take place, so it is probable that the acid medium may accelerate the rate of pigmentation by liberating the chromogen; or the process may be initiated by the slight rise of acidity due to oxidation on exposure of injured tissues to air, and this process once started may lead to the formation of coloured oxidation products of high relative acidity.

That the addition of acids and alkalis does alter the activity of enzymes is well known. Certain experimenters including EULER (1920) have determined the optimum pH for a number of enzymes and EULER's data show that this optimum varies with the substrate, or it may be that different enzymes are involved, e. g. the optimum values for butyrase range from pH 4 to pH 8, quite a considerable variation due to the substance acted upon or to the different types of butyrase. An even greater range is given by McCLENDON (1917) for protease, which has its optima from pH 1 to pH 9·7 according to the substratum (see Chapter XVI).

ATKINS, in his Recent Researches in Plant Physiology (1916) points out that five different classes of oxidases are described as occurring, according to the substances on which these act. The optimum pH for each may vary but these do not seem to have been determined.

It is not at all probable that the alcoholases, found in certain băcteria which convert ethyl alcohol into acetic acid, should give the same optimum pH as the oxidase in potato extract (1916) which according to REED is at or about pH 7, i. e. neutrality, so that we should not expect the oxidase in sunflower to show greatest activity at or near neutrality.

Acid medullary rays in underground regions of the stem and in the root may be explained by the same hypothesis as the reaction of the more acid cells in the top of the receptacle. The medullary rays serve for transference of reserves to and from the xylem and phloem and also play the rôle of storage tissue. Here sugars in the presence of oxidases may lead to increased acidity by oxidation, and to increased pigmentation, i. e. the brownish colour observed in the relatively acid medium.

SEEDS AND SEEDLINGS

As germination proceeded no changes in the reactions of the tissues of the embryo were observed.

The root cap and tip, the dermatogen of the radicle, the outer layers of the periblem and the procambial strands, the epidermis and the sub-epidermis of the cotyledons were very acid, giving a reaction as high as pH 4·4—4·0. The remainder of the embryo had an acidity of pH 5·2—4·8, so that the embryo as a whole was decidedly acid.

The embryos of seeds germinated in soil and on blotting paper showed no differences in reaction and the tissues of the germinated embryo were as acid as those of the ungerminated embryo; but in the seedling stage before the first foliage leaves were expanded a change in reaction from pH 5·2—4·8 to pH 5·9—5·6 in the cortex, endodermis, pericycle and pith above ground was observed. The other tissues remained relatively acid. The phloem when differentiated gave a pH of 5·2—4·8 which was the reaction found, with few exceptions on the more alkaline side (i. e. pH 5·9—5·6 and pH 5·9 ca.) at all stages and in all parts of the plant.

This reaction of pH 5·9—5·6 was found in the cortex, endodermis, pericycle and pith above soil of seedlings at all stages examined.

Rose (1919) records that as germination begins the reaction of the embryo changes from alkaline to acid but that the endosperm remains alkaline, in the case of *Sambucus*.

In the sunflower embryo the root cap and tip are relatively acid and they show this high acidity throughout the early seedling stages. At later stages the tips of the roots were not examined because of the difficulty in obtaining sections of these.

In older seedlings rootlets were observed passing out through the cortex. These had very acid root caps, which were yellow with B. P. B. indicating a reaction of pH $<$ 3·4; the remainder of the rootlet as seen in longitudinal section was deep blue with B. P. B. and had a pH of 5·2—4·8. This suggests that the enzyme secreted by the cap as it forces its way through the cortex is in an acid medium.

The acidity of cotyledons still enclosed in the fruit coat were compared with those expanded and free but no difference in reaction was observed. This may have been due to the reaction being masked by natural colouration.

In the very young cotyledon, before the chlorophyll was fully developed, the mesophyll gave the reaction pH 5·2—4·8 which was the figure recorded in most cases for the mesophyll in the mature cotyledon and in leaves at all stages examined, except the mature (pH 5·9—5·6).

GENERAL DISCUSSION OF REACTIONS FOUND IN SUNFLOWER

The reactions given by sunflower are interesting if we compare the fact that a $\frac{N}{100}$ HCl solution is at pH 2, and this is considered a very weak acid solution. Also, according to ATKINS, a solution of CO_2 in water nearly saturated at 25° C (and normal pressure ?) is at pH 4·8. It has been shown that even a dilute solution of ethyl alcohol has an effect on the output of CO_2 by living tissues. IRWIN and WEINSTEIN (1922) working with radish seedlings found that this alcohol decreased the production of CO_2, but at the same time organic acids were produced and it is suggested that the effect of the alcohol was an acceleration of the decomposition of certain substances with the formation of an excess of intermediate products in the form of acids.

In a recent paper (1924) E. PHILIP SMITH describes the effects of chloroform, ether and ethyl alcohol on wheat, rice and oats. The first effect was a decrease in the rate of respiration followed by an increase to a maximum and a final depression. RAY (1923) obtained different results with *Ulva*, probably due to the differences in the oxidisable material available in the tissues. SMITH also points out the possibility that the ethyl alcohol may have an effect on the permeability of the plasma membrane to carbon-dioxide so that the total result may be due to the effect of the alcohol (i) acting as an anaesthetic on respiration proper, and (ii) on the permeability of the cell to carbon-dioxide.

It is possible that the actual reactions within the cells of sunflower may be altered by the alcoholic solutions of the indicator salts but such changes, if they do take place, appear to be so small that they do not affect the range of the reaction as determined by the method used here.

Aqueous solutions of the alkali salts of B. P. B., M. R. and B. C. P. were made up according to the directions given by CLARK and the results shown by these were compared with those obtained by the use of the dilute alcoholic solutions.

No appreciable differences were detected. The aqueous solutions gave slightly less intense colouration but this does not affect the result when the 'Range-Indicator Method' is used.

Comparing young and old sunflower seedlings we find that old plants are slightly more alkaline than younger plants but variations are found at all stages and it is not surprising that such variations do occur. One expects slight fluctuations in relative acidity and alkalinity of such plants owing to the numerous factors which may operate, e. g. soil conditions may vary slightly giving different reactions but these are not likely to interfere here, as all seedlings were grown in soil from the same source.

Conditions of light, heat and moisture were more probable as disturbing factors, as it is possible for plants studied on a cold dull day to give slightly different results from those given after being exposed to heat and strong sunlight in a warm greenhouse.

Sunflowers grown out of doors did not seem to be different from those grown indoors but as the summer was wet and cold the conditions would approximate to those in winter in a slightly heated greenhouse, so that striking differences could hardly be expected.

SUMMARY

The actual acidity of sunflower (*Helianthus annuus*) was determined by means of suitable indicators. Sections of the plants at various stages and of different regions of each plant were compared.

The epidermis was relatively acid throughout, being at pH 4·4—4·0, except where the cells appeared to be injured, and here a more acid reaction i. e. pH $<$ 3·4 was obtained.

Some of the epidermal hairs were more alkaline than any other part of the plant and gave an alkalinity of pH $> 9 < 10$.

The piliferous layer and root hairs were acid and were usually at pH 4·4—4·0.

The exodermis was more acid (pH 4·4—4·0) in the early seedling stages than in older seedlings and mature plants where the reaction pH 5·2—4·8 was found.

The cortex and pith in aerial parts usually gave the reaction pH 5·9—5·6, and were sometimes at pH 5·9 ca. especially in mature plants. In subterranean parts the cortex and pith were slightly more acid and gave a pH 5·2—4·8 usually, but sometimes pH 5·9—5·6 was found.

The endodermis was more acid in underground regions than above ground. The usual reaction for the endodermis above soil was pH 5·9—5·6 sometimes pH 5·9 ca. while below soil the normal reaction was pH 4·4—4·0, sometimes pH 5·2—4·8 and occasionally as high as pH $<$ 3·4.

The pericycle underground in most cases was more alkaline than the endodermis and gave a pH of 5·2—4·8 while in some other cases pH $<$ 3·4 and pH 4·4—4·0 were recorded. Above soil the pH of the thin walled pericycle was 5·9—5·6 sometimes 5·9 ca., and corresponded in acidity with the cells of the cortex and pith.

The walls of the mature pericyclic fibres and the mature xylem fibres were among the most acid tissues of the plant. During the process of lignification a change of reaction from pH 5·2—4·8 to 4·4—4·0 and finally to pH $<$ 3·4 was observed. The walls of mature xylem vessels were acid throughout being at pH 4·4—4·0.

The phloem was more alkaline than the xylem. The reaction found most frequently, irrespective of the region of the plant examined, was pH 5·2—4·8. In mature sunflower a pH of 5·9 ca. was sometimes found.

The separate reactions given by the phloem and xylem seemed to be identical in all regions of the plant. The callus found on the sieve plates in the autumn was more alkaline than the contents of the sieve tubes and gave a reaction of pH 6·2 ca.

Cambial cells did not differ in acidity from that of the neighbouring cortical cells. Phellogen cells in the outer cortex of the stem gave interesting results. The outer cell cut off by the phellogen was more alkaline than the inner cell, the former had a pH of 5·2—4·8 while the latter was at pH 4·4—4·0.

Tissues which showed browning on exposure to air were relatively acid and gave a reaction of pH $<$ 3·4. This browning was observed in the medullary rays underground, especially in the phloem region, and in some cells at the top of the receptacle of the flower head.

The mesophyll in cotyledons and leaves appeared to be at pH 5·2—4·8 usually, and sometimes appeared to be at pH 5·9—5·6.

Young developing flower-buds gave a reaction of pH 5·2—4·8. The mature style became more acid reaching pH 4·4—4·0 (possibly pH $<$ 3·4), while the anthers matured to pH 5·9—5·6. Pollen grains had an acidity of pH 5·2—4·8. The ovary and ovule at all stages were at pH 5·2—4·8, and the corolla when developing had an acid epidermis (pH 4·4—4·0).

The reactions of the embryo ungerminated and during germination were studied. The root cap and root tip, the dermatogen and the procambial strands were more acid than the remaining tissues of the embryo. The reaction given by these was pH 4·4—4·0 while the other tissues of the embryo were at pH 5·2—4·8. No change of reaction during germination was observed. As the plant matures the parenchymatous tissues and the phloem become slightly more alkaline.

No distinct gradient of reaction for the different regions of sunflower was observed, except the difference in various tissues above and below ground.

With the exception of the more alkaline epidermal hairs all the reactions found were on the acid side of neutrality.

2. THE BUFFER SYSTEM

Although there are other buffer systems, as well as the phosphate system described below for the sunflower, they would appear to act only in tissues or cells which are below pH 5·2 in reaction. The epidermis and other acid tissues have not been examined separately, and the investigations by Miss MARTIN may be taken as applying only to the cortex and pith from which, as reported, the bulk of the expressed sap was obtained. The Buffer Index of sunflower sap is considered in Chapter XIX[1]) and Miss MARTIN's reports are therefore given below almost *verbatim*, as they appeared in „Protoplasma", Vols. I and III.

(a) *The Buffer of Sunflower Hypocotyl*

Certain experimental changes obtained in the hydrion concentration of the tissues of the sunflower hypocotyl (SMALL 1926), the disturbance which resulted from alkali-yielding bottles (SMALL 1926), and also the large natural variations which have been found amongst cells of the same tissue (MARTIN 1927, REA, SMALL 1926) all seemed to indicate the absence of strong buffers in some plant cells, and the investigation described below was carried out at the suggestion of Professor J. SMALL, as an examination of one particular case in some detail. The writer desires here to express her thanks to Professor SMALL, and also to Professor T. H. MILROY and his staff for their interest and assistance throughout the research.

INTRODUCTION

Recent researches have shown how very sensitive the various chemical and physical processes taking place in plant and animal organisms are to changes in hydrogen ion concentration.

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1) See also figure 20, Chapter XIV.

In living organisms there is present a system, sometimes a complex of interacting systems, which acts as an efficient mechanism for moderating the changes in hydrogen ion concentration so that no serious disturbance of the delicate action of the life processes may take place.

There are several base-carrying systems which are known to regulate the hydrion concentration of blood and other animal fluids, and of these the bicarbonates and the phosphates seem to be the most important. The buffer reactions of the bicarbonates with CO_2 and those of the acid and alkaline phosphates have been worked out in some detail for blood and other physiological fluids, chiefly by HENDERSON (1906 — to date). In a more recent paper (ANDREWS etc. 1924) the complex interactions of inorganic phosphates (precipitable and hydrolysable) with lactic acid and the colloidal protein systems are elucidated.

It is now generally recognised that the determining factor in many plant processes is the concentration of hydrogen ions or of hydroxyl ions present in the sap, and this concentration is regulated by the amounts present of certain substances, e. g. mixtures of weak acids and their salts, which owing to the way in which they dissociate in solution are capable of absorbing, in some cases, quite considerable quantities of acids or bases, thus buffering the reactions of the cell sap.

In plants our knowledge of buffer action is due chiefly to HEMPEL (1917), who investigated the expressed juice of succulents and concluded that in most cases malic acid and its salts interacted in such a way as to maintain the reaction of the juice within a pH range which could not injure the protoplasm.

Certain plants, in particular succulents, are highly buffered while others, e. g. *Zea mais*, are buffered only to a slight degree; and there is also the possibility that some plants are not buffered at all, at least not to any appreciable extent. In plant cells where the reaction of the sap is markedly acid organic acids and their salts are known to regulate the reactions, but up to the present no investigation of plant cells with less acid sap has been carried out (see Chapters IX and XIX).

The following research was undertaken to determine to what extent the expressed sap of sunflower (*Helianthus annuus*) was buffered and to what substances the buffer action, if any, was due.

Experiments with carbon-dioxide (REA 1926) indicated that the carbonate-bicarbonate interaction was not the effective agent in maintaining the normal reaction of sunflower sap.

It has been pointed out as a remarkable fact that "CO_2 the universal product of oxidation is the most efficient regulator of neutrality in living organisms" (HENDERSON 1913), but this conclusion was arrived at after consideration of animal organisms not of vegetable organisms. The Carbon Dioxide Balance has been shown to have important effects on ordinary plant tissues.

In succulents the formation of acids is due to incomplete respiration. The interaction of these organic acids and their salts produces marked buffer effects.

A non-succulent, such as sunflower, presents quite a different arrangement of tissues and the metabolism also differs, so that we should not expect organic acids to play an important rôle in the reactions of the cell sap of this plant. Under normal conditions the carbon dioxide of respiration of sunflower is used up in carbohydrate formation.

In the epidermis of sunflower, where photosynthesis is absent, CO_2 may accumulate and the formation of organic acids may account for the more acid reaction (pH 4·4—4·0) found; or the carbon dioxide of respiration, when slowly diffusing from the epidermal cells, may precipitate any phosphates present in solution, so that in the absence of any organic acids this sap will be unbuffered and consequently will become more acid in the presence of CO_2.

As the reaction of the expressed sap of sunflower hypocotyl is on the alkaline side of the iso-electric points of most plant proteins, any proteins present would act as acids and liberate base, but the properties of proteins as amphoteric electrolytes depend on their concentration not on their activity, as they dissociate only very slightly and do not readily form salts with weak acids or bases.

The concentration of proteins in the expressed sap was extremely low, so that the part played by such a system would be negligible, even if the proteins could carry base so far on the acid side of neutrality.

Attention was directed to the phosphate system as it was considered that inorganic phosphates, if present in dilute concentration, might be the substances buffering the sap of sunflower hypocotyl.

In all experiments with expressed sap there is the possibility that chemical changes may have taken place during the process of extraction. The reaction of expressed sap is that of a mixture of a number of cell saps where various reactions may be characteristic of each group of cells; and in addition there is the possibility or even the probability of different reactions within the same individual cell. Adsorption phenomena with the resulting phase boundaries make this condition extremely probable. In these experiments the bulk of the sap was derived from the cortex and the pith of the hypocotyl.

INORGANIC PHOSPHATE ANALYSES

The phosphate content was determined immediately after removal of the protein, to avoid complications due to bacterial or fungal action as plant extracts do not keep for any length of time.

The inorganic phosphate content was determined by Embden's gravimetric method (Embden 1921), i.e. by precipitation with a mixture of ammonium-molybdate-nitric acid solution and a concentrated solution of strychnine nitrate, (see Appendix I).

In the first few experiments the sap was weighed but in later experiments this was not considered necessary as 2 cc. sap weighed approximately 2 grams; also in these preliminary experiments the mixture of sap and protein precipitant was boiled before being filtered, but this proceeding was considered inadvisable as boiling in acid solution hydrolyses the proteins and so might liberate phosphorus from the phosphoproteins. At first varying quantities of sap (from 6 cc. to 1 cc.) were taken for the H_3PO_4 analysis but it was found that 2 cc. was a convenient quantity to work with.

First Series

H_3PO_4 from boiled extract.

0·64 grams per litre
0·708 ,, ,, ,,
0·81 ,, ,, ,,
0·62 ,, ,, ,,
0·63 ,, ,, ,,

Second Series

H_3PO_4 from unboiled extract.

0·53 grams per litre
0·501 ,, ,, ,,
0·46 ,, ,, ,,
0·54 ,, ,, ,,
0·57 ,, ,, ,,
0.56 ,, ,, ,,
0·601 ,, ,, ,,
0·531 ,, ,, ,,

All these results were taken from seedlings of approximately the same age and grown under the same conditions.

The results from the boiled extract are slightly higher than those from the unboiled extract.

The fact that the amounts of phosphoric acid obtained from the boiled extracts did not differ greatly from those of the unboiled extracts is probably due to the absence of proteins in any quantity in the cell sap.

Taking the results of the unboiled extracts we see that the inorganic phosphate content in the expressed sap of sunflower hypocotyl varied between 0·005 molar and 0·006 molar phosphoric acid.

All later experiments were carried out in the cold i. e. at room temperature.

Another lot of older, less juicy seedlings contained a little more phosphate per litre, owing possibly to their lower water content. These had inorganic phosphate concentrations corresponding to from 0·006 molar to 0·007 molar phosphoric acid. The phosphate contents of these were as follows: —

H_3PO_4 in grams per litre
0·64
0·67
0·68
0·65
0·66

REACTION AND BUFFER VALUES OF EXPRESSED SAP

The buffer effects of several samples of expressed sap were then determined and these results showed that the buffer value of sunflower sap, in terms of molar H_3PO_4, corresponded closely to that of the actual concentration of inorganic phosphates present in solution in the cell sap.

Some samples of sap were diluted with an equal quantity of neutral water, while other samples were tested for buffer values without previous dilution. The buffer values of the undiluted sap in terms of molar phosphoric acid were as follows: —

H_3PO_4
0·0069 M.
0·0068 ,,
0·0069 ,,
0·0058 ,,
0·0065 ,,
0·0065 ,,

The buffer values of the diluted sap were as follows: —

H_3PO_4
0·0064 M.
0·004 ,,
0·007 ,,
0·0065 ,,
0·0055 ,,
0·0066 ,,

As these results correspond closely we conclude that dilution with an equal quantity of neutral water did not alter the buffer value of the sap.

Determinations of the reactions of sap and of phosphate solutions were made by matching the colour, before and after each addition of acid or alkali, with that of standard solutions of known pH made up from B.D.H. universal buffer solution.

For this purpose the liquid was introduced into small tintometer bottles with two flat sides, as it is considered that this method gives more accurate results than judging the colours in test tubes.

EXPERIMENTAL

EXTRACTION AND DEPROTEINISATION OF SAP

The sap of sunflower hypocotyl was expressed by crushing the tissues in a small press. This expressed sap was filtered and centrifuged.

The centrifuged sap contained some colouring matter so that care was necessary in determining the actual reaction.

Any proteins present in the expressed sap were removed by a 10 per cent solution or a 3 per cent solution of trichloracetic acid. In some cases the proteins were precipitated with sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) 10 per cent solution and H_2SO_4 in normal solution.

Normal H_2SO_4 was used in preference to $^2/_3$ n H_2SO_4 owing to the fact that excess acid prevented precipitation, as calcium phosphate, of any calcium present, which would have lowered the apparent phosphate content.

The filtrate obtained by this method was always turbid and required refiltering.

This is the method, given by MILROY (1921) for the preparation of protein free blood filtrates, slightly modified to suit the substance being deproteinised.

Trichloracetic acid gave a clear filtrate which gave more reliable results than the sodium tungstate and H_2SO_4 filtrate which in some cases contained traces of proteins or their hydrolytic products.

SPECIMEN DETAILS OF SAP SAMPLE NO. 4. (BELOW)

A series of experiments were carried out in which the buffer value of the sap was determined first, then another part of the same sample was analysed for inorganic phosphates.

One experiment is given in detail below.

Expt. 1. (1) Reaction determinations: The reaction of the expressed sap at room temperature was pH 5·6. At this reaction the ratio of K_2HPO_4 to KH_2PO_4 corresponds to per mol phosphoric acid $\frac{36{\cdot}4}{963{\cdot}6}$ in millimols.

2 cc. of this sap were taken and 0·5 cc. NaOH 0·005 M. (1·25 millimols per litre) added and the reaction again determined, when the pH was found to be 6·4.

At this reaction the ratio $\frac{K_2HPO_4}{KH_2PO_4}$ becomes $\frac{252}{748}$, an increase of 215·6 millimols per mol phosphoric acid and therefore the 1·25 millimols base added correspond to 0·0058 M. H_3PO_4, i. e. a 0·0058 molar phosphate solution of pH 5·6 would show a shift

From these results it seems quite clear that the buffer effect in the sap of the sunflower hypocotyl between pH 5·6 and pH 6·8 is in fact due to the inorganic phosphates present at a very low concentration.

Parallel experiments were carried out with undiluted sap and with sap diluted with an equal quantity of neutral water. The buffer capacity of equal amounts of sap corresponded in each.

The results of two such experiments are given below: —

I. Diluted sap

	NAOH (·005 M.)	pH	$\frac{K_2HPO_4}{KH_2PO_4}$	Base equivalents per litre	M' mols base	Buffer value
Indicator B. C. P.						
2 cc. diluted juice .	0	5·6	—	—	—	—
2 " " " .	0·2 cc.	6·0	—	—	—	—
2 " " " .	0·4 "	6·2	—	—	—	—
Indicator B. T. B.						
2 cc. diluted juice .	0	5·6	—	—	—	
2 " " " .	0·2 cc.	—	—	—	—	
2 " " " .	0·4 "	6·2	—	—	—	
2 " " " .	0·6 "	6·6	—	—	—	
2 " " " .	0·8 "	7·0	—	—	—	—
2 " " " .	1·0 "	7·4—7·2	—	—	—	—
Indicator P. R.						
2 cc. diluted juice .	0	5·6	$\frac{·364}{9·636}$	753·6	0	
2 " " " .	0·8 cc.	7·0	$\frac{5·92}{4·08}$		2·0	
2 " " " .	1.0 "	7·4	$\frac{7·9}{2·1}$		2 5	·0033 M. H_3PO_4

This corresponds to a buffer value for undiluted juice of ·0066 M. phosphoric acid between pH 5·6 and pH 7·4.

II. Undiluted sap

	Na OH (·005 M.)	pH	M'mals base aded	$\frac{K_2HPO_4}{KH_2PO_4}$	Base equivalents per litre	Buffer value
Indicator D. E. R.						
2 cc. Juice	0	5·6	0	·364 / 9·636	215·6	·0069 M. HP_3O_4
2 ,, ,, ...	0·2 cc.	5·8	0·5	·75 / 9·25		
2 ,, ,, ...	0·4 ,,	6·2	1·0	1·72 / 8·28		
2 ,, ,, ...	0·6 ,,	6·4	1·5	2·52 / 7·48		
Indicator B. C. P.						
2 cc. Juice	0	5·6	0	·364 / 9·636	215·6	0·0069
2 ,, ,, ...	0·2 cc.	5·8	0·5	·75 / 9·25		
2 ,, ,, ...	0·4 ,,	6·2	1·0	1·72 / 8·28		
2 ,, ,, ...	0·6 ,,	6·4	1·5	2·52 / 7·48		
Indicator B. T. B.						
2 cc. Juice	0	5·6	0	·364 / 9·636	215·6	0·0069
2 ,, ,, ...	0·2 cc.	—	0·5	—		
2 ,, ,, ...	0·4 ,,	6·2	1·0	1·72 / 8·28		
2 ,, ,, ...	0·6 ,,	6·4	1·5	2·52 / 7·48		

The addition of 0·6 cc. i. e. 1·5 millimols of ·005 M. HCl to the sap at pH 6·4 shifted the reaction back to the original pH 5·6, showing that the buffer value had not changed while the experiment was proceeding.

CARBON DIOXIDE EFFECTS ON EXPRESSED SAP AND ON PHOSPHATE BUFFER SOLUTIONS

The effects of different percentages of carbon dioxide on sunflower sap and on a dilute solution of KH_2PO_4 of molar concentration approximately equal to that found in expressed sap were determined.

The reaction of this 0·007 M. KH_2PO_4 was pH 5·0.

In order to test the buffer value of this phosphate solution within the same range as that in which the expressed sap was examined, alkali (·005 M. NaOH) was added until the reaction was pH 5·6.

To obtain known percentages of carbon dioxide the small inverted burette from a GANONG's photosynthometer (capacity about 20 cc.) was used. This burette was first filled with mercury (which had been previously cleaned by squeezing through clean chamois leather), then inverted and a known amount of mercury was displaced by air. Then the required amount of washed carbon dioxide was bubbled in from a Kipp apparatus, displacing its own volume of mercury at atmospheric pressure. In this way, by varying the amounts of air and carbon dioxide allowed to displace the mercury, a known percentage of carbon dioxide could be obtained.

After bubbling in the carbon dioxide the taps were closed. At one end of the burette a small tube of about 2 cc. capacity, containing one drop of indicator solution and the liquid to be experimented with, was attached by rubber tubing and a clip. The clip was then removed and the mercury was allowed to come down into the tube, displacing the liquid upwards into an atmosphere of carbon dioxide of known percentage.

One cubic centimetre of liquid was tested in this way, while the remainder was left in the tube and served as a comparison with the liquid acted upon by the carbon dioxide.

After closing the clip, the liquid was shaken up with the gases in the burette and the acidified liquid was transferred to the tintometer bottles, where the colour was matched with that of a solution of known pH. In this way the reactions of the expressed sap of sunflower hypocotyl and of a dilute phosphate solution in the presence of varying percentages of carbon dioxide were determined.

The results of these experiments are tabulated below: (p. 217).

Table XIV

Reactions of expressed sap of sunflower hypocotyl, within creasing percentages of CO_2

% CO_2	0	4	5	6	7	8	9	10	20	30	40	50	60	70	80	90
pH	5·6	5·6	5·6—5·4	5·4	5·4—5·2	5·2	5·2	5·0—4·8	4·8	4·6	4·6—4·4	4·6—4·4	4·4—4·2	4·2	4·2—4·0	4·0 3·8

Table XV

Reactions of a dilute phosphate solution, of molar concentration approximately equal to the phosphate content of sunflower sap, with increasing percentages of CO_2

% CO_2	0	4	5	6	7	8	9	10	20	30	40	50	60	70	80	90
pH	5·6	5·6	5·4	5·4—5·2	5·2	5·2	5·2—5·0	5·0—4·8	4·8—4·6	4·6	4·6	4·6—4·4	4·4—4·2	4·2	4·2—4·0	4·0

In all CO_2 experiments there must be an error due to the escape of CO_2 as a gas. To avoid this error as far as may be it is necessary to pour the liquid into the bottle as quickly as possible and close the mouth of the bottle with the glass stopper.

Acidified sap at pH 4·8 was allowed to remain overnight in an unstoppered bottle, when the pH was found to have gone back to the original i. e. pH 5·6.

Of course other factors, in addition to the escape of CO_2 gas, may have come into action.

The expressed sap of sunflower hypocotyl gave at room temperature a reaction value of pH 5·6.

At this reaction the ratio $\frac{K_2HPO_4}{KH_2PO_4}$ is $\frac{\cdot 364}{9{\cdot}636}$.

At pH 5·8 the ratio is $\frac{\cdot 75}{9{\cdot}25}$, so that normally the reaction of the sap is near the unbuffered range, on the acid side, of the phosphate system. Below pH 5·2 all the inorganic phosphates present in solution are converted into KH_2PO_4. *Beyond pH 5·2, therefore, carbon dioxide may exert its full acidifying influence, acting as it would on a completely unbuffered fluid.*

Under normal conditions the reaction of the sap is maintained at pH 5·6 by removal of the carbon dioxide of respiration by photosynthesis or by diffusion.

If the tissues of the sunflower hypocotyl were highly buffered then carbon dioxide could not have produced such effects as those found, but with a sap buffered only with a very dilute solution of phosphates then carbon dioxide, if present in sufficient concentration, can produce shifts from the normal reaction and these reaction swings may cause the changes which are found.

SUMMARY

The inorganic phosphate content in different samples of sap varies from 0·005 M. to 0·007 M. phosphoric acid and the buffer values of this sap, in terms of molar phosphoric acid, corresponded very closely to the actual phosphate concentrations.

The normal reactions of the sap of sunflower hypocotyl are, therefore, only slightly buffered and this small amount of buffer action is due to a correspondingly dilute concentration of inorganic phosphate present in the sap.

Heating the sap over-night at a temperature of 30° C did not increase the inorganic phosphate content.

In an atmosphere of CO_2 of concentrations ranging from 5 per cent upwards the normal reaction pH 5·6 of the sap was changed to reactions of higher acidity. Below 5 per cent the CO_2 did not affect the hydrogen ion concentration within a pH range of 0·2, while above 5 per cent a progressive series of reactions of decreasing pH values was obtained with increasing concentrations of carbon dioxide.

5 per cent carbon dioxide shifted the reaction from pH 5·6 to pH 5·4.

90 per cent carbon dioxide shifted the reaction of the sap from pH 5·6 to pH 4·0—3·8.

No determinations were made with 100 per cent carbon dioxide.

The more acid epidermis (pH 4·4—4·0) of sunflower presents interesting possibilities, and the nature of the physical and chemical processes to which this higher acidity is due has not been elucidated.

Addendum

(One Experiment only. Further work required)

A large quantity of sap was left overnight at a temperature of 30° C. A brown precipitate settled out leaving a clear extract above. This extract was filtered, then its reaction and buffer value were determined at room temperature. The reaction of this extract was pH 5·2 and the buffer value between pH 5·2 and pH 6·4 corresponded to that of a 0·012 molar phosphoric acid solution.

The inorganic phosphate content of this extract was not determined.

At pH 5·2 the ratio of $\frac{K_2HPO_4}{KH_2PO_4}$ in a 0·0068 M. H_3PO_4 solution is $\frac{\cdot 000034}{\cdot 006766}$. The total base content of this phosphate system is 6·834 millimols.

At pH 6·4 the ratio in 0·0068 molar H_3PO_4 is $\frac{\cdot 0017136}{\cdot 0050864}$.

The total base content is 8·5136 millimols, giving an increase of 1·6796 millimols.

The addition of 1·6796 millimols of base to a 0·0068 molar phosphoric acid solution at pH 5·2 would change the reaction to pH 6·4.

The clear extract required 2·5 millimols base to swing its reaction from pH 5·2 to pH 6·4, so that acid groups other than the 0·0068 molar H_3PO_4 must have been present in sufficient quantity to absorb approximately 8 millimols base.

The clear extract may have contained phosphates corresponding to more than 0·0068 M. H_3PO_4, (this being the actual phosphate content of another sample of the same juice which was heated and in which the clear extract and precipitate were shaken up and analysed together), if all the inorganic phosphates present remained in solution in the clear portion of the sap.

The source of this higher acidity might be due to hydrolytic products of proteins or other organic substances present in the sap.

Bacterial and fungal action may also have played a part.

Further investigations of the effects of heating the sap are necessary.

(b) The Buffers of Sunflower Stem and Root

INTRODUCTION

The actual acidities of the tissues of Sunflower stem and. root are recorded above. We will only briefly refer to these here.

In these tissues individual variations do occur, but these are of a highly restricted nature, e. g. the reaction of the parenchymatous tissues varies between the ranges pH 5·2—4·8 and pH 5·9 ca.; pH 5·9—5·6 being the reaction range found most frequently.

These slight variations may be due to one or more of the numerous factors which are known to affect the relative acidity of plant tissues. Soil conditions, light, heat, etc., may be responsible, or the variations may be due to internal factors the action of which is still uncertain.

Apart from these slight natural variations, examination of the observations referred to above reveals a remarkable constancy in the pH values maintained throughout the life-history of the plant, and that not only in the individual, for on comparing a number of plants of the same species we find the same pH values occurring again and again. Having studied one of any species of plant, we may, with a very fair degree of accuracy, state the characteristic pH of each tissue of any number of that species (Chapter X) provided, of course, that external conditions do not vary to such an extent as to upset the normal life-processes within these plants (Small 1920). The reaction values have also been shown to be remarkably constant in some plant tissues throughout the year (Chapter XI).

Consideration of recent work on plant and animal tissues leaves no doubt that this constancy is, to some extent at least, due to buffer action within the cells.

The importance of buffer action in the life of organisms has been dealt with in some detail by several workers and has been emphasised especially in the case of the body fluids of animals. In the plant kingdom succulent plants, where the degree of buffer action is high, have been made the chief subject of study (HEMPEL 1917).

Owing, perhaps, to the small quantities of the substances responsible for the comparatively slight amount of buffer action found in some non-succulent plants, very few of these have been studied in any detail, and only in a few cases has the buffering of the juice been traced to some substance or substances within the sap (see Chapter XIX).

In order to understand how, under ordinary circumstances, the relative acidity of any plant cell does not shift far from its normal reaction, it is necessary to determine what the buffering substances are, the concentrations of these present, and the range in which these may be effective.

Mixtures of the acid and the alkaline salts of phosphoric acid buffer between pH 5·2 to pH 8 approximately, while other substances are effective at higher and lower pH values.

It has been shown above that in the case of the *hypocotyl* of Sunflower seedlings a small concentration of inorganic phosphates was present in sufficient quantity to account for the buffer value of the expressed sap as determined by titration with alkali, between pH 5·6—6·8.

Titration was carried out within arbitrary limits which did not vary far beyond the range of hydrion concentration found within the tissues of the plant.

In the present investigation stems and roots of mature Sunflower plants were examined, by the same methods as were used in the study of the buffer processes in the expressed juice of the *hypocotyl* of the seedling.

EXPERIMENTAL

Stems of mature Sunflower (*Helianthus annuus*) were cut off slightly above soil level. The leaves and the stem tip were cut off and the juice of the stem was expressed by crushing the tissues in a small press.

In a few cases this expressed juice was centrifuged, but, as centrifuging with a small hand centrifuge was rather a slow process, in all the later experiments the sap was filtered through a **Buchner** funnel lined with filter paper and containing asbestos which had been previously well washed with neutral water and sucked dry with a filter pump.

This procedure cleared the sap of scraps of tissue and the filtered sap was practically devoid of colouring matter.

As this method of clearing the expressed sap is much quicker than centrifuging, possible physico-chemical effects such as oxidation on exposure, etc. are minimised.

The expressed sap was then utilised immediately for inorganic phosphate analysis and determinations of buffer values.

The roots were cut off and well washed under running tap water. The finer particles adhering to the roots were brushed off with a camelhair brush. When as free as possible from foreign matter the roots were washed with distilled water, then with neutral water, after which they were dried by soaking off the moisture with blotting paper.

This procedure must have left some moisture adhering to the roots but this does not seriously affect the results as the comparison of the actual phosphate concentration with the degree of buffer action is more important than the mere measurement of phosphate content of undiluted sap.

The buffer value and the phosphate concentration will be equally altered by dilution and hence the results will still be comparable.

Since dilution does not change the ratio of acid salt to normal salt in dilute concentration, the pH value of the sap on dilution should not shift to any appreciable extent.

After the roots were cleaned, the tissues were crushed and the expressed sap was filtered by the same method as was used in the case of the stem tissues.

In all experiments with expressed sap it is essential to compare the juice of corresponding parts of plants as different parts may differ in acidity, e. g. the parenchymatous cells of the root in Sunflower had slightly more acid cell contents than the corresponding tissue in the stem. In these stems and roots no continuous gradient of reaction was observed. With the exception of the underground part of the stem which was avoided in this investigation, the upper, middle and lower portions of these regions showed the same pH values, so that the sap expressed from the stem region or the root region as a whole is not a mixture of cell-saps varying in reaction from region to region. By using the sap expressed from stem and root separately we get a fair approximation to the true state of the conditions in the sap of stem and root as far as the phosphate factor is concerned.

Sap reactions were determined by matching the colours given by suitable indicators with standard solutions of known pH, using small flat-sided bottles in all cases. The indicators used were Methyl Red, Diethyl Red, Bromo-cresol Purple, Bromothymol Blue and Phenol Red.

All experiments were carried out at room temperature. EMBDEN's Phosphate Method as given in Appendix I was used throughout.

BUFFER VALUES AND INORGANIC PHOSPHATE ANALYSES OF EXPRESSED SAP OF SUNFLOWER STEM

Experiment I

The reaction of the sap was pH 5·8.

Using undiluted sap the following shifts in reaction on addition of 0·005 M NaOH were obtained.

The buffer values are expressed in terms of molar phosphoric acid.

2 c.c. sap were used for each determination.

The results are tabulated below.

Original pH	c. c. sap	c. c. NaOH added	pH after addition of alkali	Buffer values
5·8	2	0·1	6·0—5·8	—
5·8	2	0·2	6·4—6·2	—
5·8	2	0·3	6·4	0·0042
5·8	2	0·5	6·6	0·0044

The amount of H_3PO_4 present in another 2 c.c. portion of this sap was 0·43148 grammes per litre or 0·0044 M, as determined by precipitation and gravimetric analysis.

On looking at the table above we see that the addition of 0·5 c. c. NaOH 0·005 M, in other words 1·25 millimols base, to the juice at pH 5·8 caused a reaction swing to pH 6·6.

At pH 5·8 the ratio $K_2HPO_4 : KH_2PO_4$ is $\frac{75}{925}$ expressed in millimols per litre. At pH 6·6 the ratio $K_2PHO_4 : KH_2PO_4$ becomes $\frac{356}{644}$ so that a molar phosphate solution would require the addition of 281 millimols base per litre to shift its reaction from pH 5·8 to pH 6·6. This shift in sap reaction was caused by 1·25 millimols base; therefore, the system behaved as a 0·0044 molar solution and this was the actual concentration of inorganic phosphate present in the sap, as determined by gravimetric analysis.

A few other experiments are given below.

Experiment II

In this case each c.c. sap was diluted to twice its volume with neutral water.

Original pH	c. c. dil. sap	c. c. NaOH	pH	Buffer value
5·8	2	0·1	6·2	0·0051
5·8	2	0·2	6·6- 6·4	-
5·8	2	0·3	6·6	0·0053
5·8	2	0·4	6·8	0·0050
5·8	2	0·5	7·0	0·0048

To an undiluted 2 c. c. portion of this sap the addition of 0·2 c. c. NaOH 0·005 M gave a shift in reaction to pH 6·2 corresponding to 0·0051 M H_3PO_4 i. e. a phosphate solution containing 0·0051 M H_3PO_4 would change in reaction from pH 5·8 to pH 6·2, on addition of 1 millimol base per litre.

The actual concentration of H_3PO_4 present was 0·49044 grammes per litre, which is equal to a 0·0050 molar solution.

Experiment III

In this experiment the sap was again diluted to twice its volume, with neutral water.

Original pH	c. c. dil. sap	c. c. NaOH	pH	Buffer value
5·8	2	0·1	6·4—6·2	—
5·8	2	0·15	6·4	0·0042
5·8	2	0·5	7·2	0·0040

H_3PO_4 determined by gravimetric analysis = 0·0039 M.

Experiment IV

Sap diluted to twice its volume with neutral water.

Original pH	c. c. dil. sap	c. c. NaOH	pH	Buffer value
5·8	2	0·1	6·2	0·0051
5·8	2	0·15	6·4	0·0042

Average buffer value = 0·0046.

Actual concentration of H_3PO_4 present was 0·0047 M.

From the above results we conclude that inorganic phosphates are present in solution in the expressed sap of Sunflower stem, in sufficient concentration to account for the buffer action found within the sap.

SUNFLOWER ROOT

Owing to the considerable time necessary to obtain sap from Sunflower roots only a few c. c. sap were available for each experiment.

The results from three experiments are given below.

Experiment I

Sap diluted to twice its volume with neutral water.

The reaction of this expressed sap was pH 5·0.

Original pH	c. c. dil. sap	c. c. NaOH	pH	Buffer value
5·0	2	0·1	5·6	—
5·0	2	0·2	6·0—5·8	—
5·0	2	0·3	6·2	0·0070
5·0	2	0·4	6·4	0·0069

The addition of 0·1 c. c. NaOH brought the reaction of the sap from pH 5·0 to pH 5·6, and 0·4 c. c. swung the reaction to pH 6·4.

As pH 5·0 is on the acid side outside of the range of the phosphate buffer system we consider the reaction swing between pH 5·6 and pH 6·4.

At pH 5·6 the ratio $\frac{K_2HPO_4}{KH_2PO_4} = \frac{36\cdot4}{963\cdot6}$

At pH 6·4 the ratio $\frac{K_2HPO_4}{KH_2PO_4} = \frac{252}{748}$.

Between these two reaction points 215·6 millimols base per litre are required to give this shift in a molar solution. 0·3 c. c. NaOH were added to 1 c. c. sap (or 2 c. c. diluted sap) which is equal to 0·15 millimols base per litre. Therefore the buffer value of the sap, in terms of molar phosphoric acid was 0·0069 M.

The concentration of inorganic phosphates present was 0·0074 M.

Experiment II

The reaction of 2 c. c. sap on the addition of 0·2 c. c. NaOH, changed from pH 5·6 to pH 6·2. This reaction shift calculated in terms of molar phosphoric acid indicates a buffer value of 0·0037 M.

The remainder of the sap was diluted to twice its volume with neutral water and titration with alkali gave the following results:

Original pH	c. c. sap	c. c. NaOH	pH	Buffer value
5·6	2	0·2	6·2	0·0037 M
5·6	2	0·3	6·4	0·0034 M
5·6	2	0·4	6·6	0·0031 M
5·6	2	0·5	6·8—6·6	—

The average buffer value from the above table is 0·0034 M. The concentration of inorganic phosphates was 0·0033 M which figure corresponds fairly closely to the degree of buffer action present in the sap.

Experiment III

The reaction of the expressed sap was pH 5·6.

The addition of 0·2 c. c. NaOH 0·005 M brought the actual acidity of the sap to pH 6·2 while the addition of 0·4 c. c. NaOH to another 2 c. c. sample of the sap shifted the reaction to between pH 6·6 and pH 6·4 as is shown in the following table.

Original pH	c. c. sap	c. c. NaOH	pH	Buffer value
5·6	2	0·2	6·2	0·0037
5·6	2	0·4	6·6	0·0031
—	—	—	6·4	0·0046
5·6	2	0·4	6·6	0·0031

The inorganic phosphate content of a sample of the sap was 0·0034 M.

Other determinations of phosphate content and buffer values gave similar results showing, that in the root as in the stem, the buffering of the sap, between the reaction points determined, is due to the small amount of inorganic phosphates dissolved therein.

The amount of inorganic phosphate present in the sap of Sunflower stem and root is sufficient to account for the buffer values found, within the limits of the reaction of the expressed sap to the pH values mentioned above.

This does not exclude the possibility that there are substances within the plant which may act as buffers at higher or lower degrees of acidity or alkalinity.

Hempel (1917) on titrating the juice of succulent plants between pH 6·81 and pH 9·3 (litmus and phenolphthalein transition points respectively) found that some substances were present which were capable of acting as acids at these low concentrations of hydrogen ions. These substances do not normally act as acids within the plant since the cell-sap reaction of these comparatively strongly buffered plants is maintained at a much lower pH value by a buffer system consisting of mixtures of organic acids and their salts. Of these substances aluminium hydroxide, a typical inorganic ampholyte was chiefly responsible for the buffer action between pH 6·81 and pH 9·3.

It may be that in Sunflower sap some such amphoteric substances e. g. amino-acids and their derivatives which are known to have some degree of buffer action at pH values on the alkaline side of neutrality are present; but we are here considering the normal conditions within the plant and for this reason titration beyond neutrality (pH 7·0) was not carried out.

CONCLUSION

Throughout the normal range of reaction for cortex and ground tissue, the juice of the stems and roots of mature Sunflower plants is buffered, as in the hypocotyl, by a small concentration of inorganic phosphates present in solution in the cell sap.

These investigations by Miss MARTIN demonstrated quite clearly that buffer action in at least some non-succulent plants is very small within the natural range of pH as found in the tissues. By accounting completely in an accurate quantitative manner for the buffer action found by the actual phosphate content as determined experimentally, Miss MARTIN opened up the field for this type of work in plants, and at the same time demonstrated how ill-founded had been the previous generalisations based upon a supposed similarity in the degree of buffer action for plants and animals. Fortunately the first example chosen presented a simple solution but that there are other problems will be made clear in the next chapter.

CHAPTER XIII

THE BROAD BEAN (VICIA FABA)

TISSUE REACTIONS AND BUFFER SYSTEMS

These are given below almost exactly as reported upon in „Protoplasma", Vols. II and III by REA and SMALL (1927) as to tissues, and by MARTIN (1927) as to buffer-systems.

1. THE TISSUE REACTIONS OF VICIA FABA

Some of our earliest observations clearly demonstrated that the sunflower and the broad bean differed considerably in the differentiation of their tissues with regard to hydrion concentration. One of the outstanding features of the sunflower is the marked acidity of the epidermis and this character is absent from the broad bean. Attention was, therefore, directed to the tissues of *Vicia faba*. These tissues were examined as to their hydrion concentration throughout the plant and at various stages in the life of the plant. The account which follows details the results of our observations on this species.

MATERIAL

It has long been known that the seed of the broad bean contains an indicator which changes from green (acid) to yellow (alkaline) according to the pH of the medium. Both the green and white (or brown) varieties were, therefore, examined. The material of the green variety was the Green Harlington Windsor, and that of the white or brown variety was the Giant Windsor Bean (White), both supplied by Messrs. Dickson of Belfast. In the Tables of Results given below square brackets [ ] indicate the data obtained for the white variety *where these differ* from the corresponding data for the green variety. Where the results were the same, as in most cases, a single letter or set of letters is given without the square brackets. The use of ordinary brackets indicates, as in previous chapters, that the reaction given in the brackets is not considered the normal one for that tissue.

Seeds were examined after being soaked in neutral water overnight. Seeds were germinated on wet blotting paper and in soil, and examined. Plants were grown in pots of soil in an unheated greenhouse with a southern aspect.

METHOD

All the tissues of at least two plants of each variety were examined at each stage. Three sections of each region were used for each indicator and these were examined according to the R. I. M. Comparable stages of development were obtained by taking plants matured to the extent of expanding one, two, three, etc., foliage leaves, the last stage examined being that of a maturely developed flowering plant.

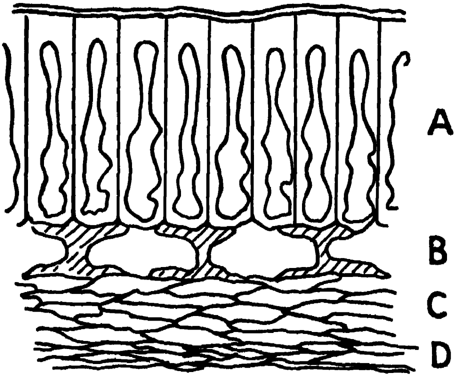

Fig. 18. Section across the integuments of the broad bean. *A* — outer layer, *B* — middle layer, *C* — inner layer, *D* — innermost layer.

RESULTS

SEED. (Table I.) In the ungerminated soaked seed all the tissues of the embryo, except the cotyledons (pH 5·6), and the dermatogen of the radicle (pH 4·4—4·0), are at pH 5·2—4·8. The outer layer of the integuments is pH 4·4—4·0, the middle and innermost layers are pH 5·2—4·8, and the first inner layer of pH 5·6. There is obviously more differentiation in the seed coats than in the embryo. No difference was observed between the reactions of the seeds of the two varieties when ungerminated.

Table I

SEED	PLUMULE							ROOT							INTEGUMENTS			
	Epidermis	Sub-Epidermis	Cortex	Pro-Camb. Tiss.	Pith	Leaf Traces	Young Leaves	Root Cap	Root Tip	Dermatogen	Periblem	Plerome	Pro-Camb. Tiss.	Cotyledons	Outer Layer	Middle Layer	Inner Layer	Innermost Layer
Ungerminated.	e	c	e	e	—	e	e	e	c	h	e	e	e	c	h	e	c	c
Germ'd in Soil	e	e	c	eh	e	e	e	e	e	h	e	e	h	c	h	e	c	h [e]
Germ'd on B.P.	e	c	e	h	e	e	e	e	e	h	e	e	h	c	h	c	c	h [e]

Table II

SEED-LING Radicle 4 cms. long	PLUMULE										INTEGUMENTS			
	Epidermis	Sub-Epidermis	Cortex	Endodermis	Pro-Camb. Tiss.	Phloem	Xylem	Pith	Leaf-Traces	Cotyledons	Outer	Middle	Inner	Innermost
in Soil .	e(e)[e]	—-[e]	ee[e]	c	h	e	h	ee[e]	c	c	h	e	e[d]	h[e]
on P. B..	c	c	c	—	h	—	—	c	e	c	h	c	c	h[e]

Radicle 4 cms. long	L. S. RADICLE						T. S. RADICLE 2 cms. from tip							T. S. RADICLE near cotyledons						
	Root Cap	Root Tip	Dermatogen	Periblem	Plerome	Vasc. Tiss.	Hairs	Pil. Layer	Cortex	Endodermis	Phloem	Xylem	Pith	Pil. Layer	Cortex	Endodermis	Pericycle	Phloem	Xylem	Pith
in Soil . . .	he	e	h	e	e	h	e	h	ee	e	c	h	ee	h	ee	e	e	e	h	ee
on B. P. . .	c	c	h	e	c	h	e	h	ee	e	e	h	ee	h	e	e	e	e	h	e

GERMINATION. (Tables I—III.) The only changes which occurred in the embryo during the early stages of germination, up to the time when the radicle was about 4 cms. long, were increased acidity in the procambial tissues of both plumule and radicle, from pH 5·2—4·8 to pH 4·4—4·0. The reaction of the cotyledons rose during the later seedling stages to pH 5·9 (Table III).

Table III

SEEDLING	Plumule 2·5 cms. long	1st Foliage Leaf Expanded	2nd Foliage Leaf Expanded	3rd Foliage Leaf Expanded	4th Foliage Leaf Expanded	5th Foliage Leaf Expanded	6th Foliage Leaf Expanded	Mature plant
Stages	I	II	III	IV	V	VI	VII	VIII
COTYDEDON								
T. S. Near Root . . .	c	c	c	c[c]	a	a	a	a
INTEGUMENTS								
Layers — Outer . . .	h	hk	hk	h	—	—	—	—
Middle . .	e	c	c	e	—	—	—	—
Inner . . .	c	e[c]	c[d]	c[d]	—	—	—	—
Innermost .	h[e]	e[h]	h	h[c]	—	—	—	—

INTEGUMENTS. There was an increase of acidity, from pH 5·2—4·8 to pH 4·4—4·0, in the innermost layer of the integuments during the early stages of germination (Table I). The only differences observed, when the white variety was compared with the green variety, were lesser acidity in the innermost layers of the integuments after germination, white **e** instead of green **h**. The reaction of the integuments was followed up to the period of the expansion of the third foliage leaf. The first inner layer (*C*, Fig. 18) tended to become more acid in both varieties; the middle layer remained constant (pH 5·2—4·8) throughout; and the innermost layer was recorded at all stages as **h** or **e** in bith varieties (Table III).

SEEDLING. No significant difference was observed in the tissue reactions of beans germinated in soil and those germinated on damp blotting paper; nor was any significant difference noted in the tissue reactions of the green and white varieties in the later seedling stages, except in the epidermis of the stem and also a repetition of the **e** for **h** in the innermost layers of the integuments (Table II). Apart from changes apparently coincident with lignification, the only noteworthy change in the later seedling stages of the embryo is the decreased acidity (**c** to **a**) of the cotyledons in both varieties after the expansion of the fourth foliage leaf.

TISSUES OF THE STEM. (Table IV)

Throughout this investigation considerable variation was found in certain tissues from plant and from stage to stage of the growth; and it seems that the pH conditions in many of the tissues are not so stable as those in the sunflower (cp. Chapter XII).

EPIDERMIS. In the seedling stages the epidermis was normally pH 5·6 in the green variety, but in the white and occasional plants of the former variety this tissue was more acid, pH 5·2—4·8. In the later stages the epidermis is mainly more acid near the tip (e) than lower down (c); the pH 5·6 persisting even below the surface of the soil, where the change seen in the sunflower (p. 192) does not take place.

SUB-EPIDERMIS. This tissue is normally similar to the epidermis in its reaction, except in the more mature stages of the middle region (half-way up) where it tends to be the same as the cortex and slightly less acid, a, than the epidermis, c.

CORTEX. Except in the seedling stage and near the apex of the mature plant where pH 5·2—4·8 occurs, the cortex is generally towards the less acid side, near the top at pH 5·6 and elsewhere slightly more so pH 5·9.

COLLENCHYMA. Where it occurs this tissue is normally of pH 5·2—4·8.

ENDODERMIS. Usually the endodermis is also of pH 5·2 to 4·8, but the observations for the middle region of the stem show a series of erratic changes from stage to stage which require further investigations. Some of the indicators do not react with certain stages of this tissue.

PERICYCLE. This tissue is similar in reaction to the endodermis, but again in the middle region of the stem there are unexplained variations. It should also be noted that in the middle and lower regions of the stem lignification of this tissue sets in and the reaction of the wall becomes pH 4·4—4·0. In the mature plant a few fibres were found at the base of the stem, which had very acid (k) walls.

PHLOEM. The general reaction here also was pH 5·2—4·8, but in the lower part of the stem the sieve-tubes at all stages were more acid (pH 4·4—4·0) than the rest of the phloem.

Table IV

STEM		Epidermis	Sub-epidermis	Cortex	Collenchyma	Endodermis	Pericycle	Phloem	Sieve-Tubes	Cambium	Xylem	Pith
Stages as in Table III												
T. S. Near Apex	I	—	—	—	—	—	—	—	—	—	—	—
	II	e	c	c	c	c	e	c	—	c	h	a
	III	e	c	c	c	c	c	c[c]	—	c	h	a
	IV	e[c]	e[c]	e[ac]	c	e[c]	e[c]	e[c]	—	c[c]	h	c[a]
	V	c	e	c	e	c	c	c	—	c	h	c[a]
	VI	e	e	c	c	c	e	c	—	c	h	a
	VII	e	e	c	e	e	e	c	—	c	h	a
	VIII	e	e	c	e	e	e	c	—	c	h	c
T. S. Half Way Up	I	—	—	—	—	—	—	—	—	—	—	—
	II	c	c	c	—	c	c	e	—	c	h	a
	III	c	c	a	—	a[c]	a[c]	c	—	c	h	a
	IV	e[c]	e[c]	e[c]	e	e[c]	c[c]	c[c]	—	c[c]	h	c[a]
	V	c	c	a	e	c	c	c[c]	—	c[c]	h	a
	VI	c	a	a	c	d	d	d	—	—	h	a
	VII	c	a	a	e	d	d	d	—	—	h	a
	VIII	(c)c	a	a	c	c	h[1]	d	—	—	h	h outer a inner
T. S. Just Below Soil	I	c(c)	c	a	—	c	c	c	h	—	h	a (c)
	II	c	c	a	—	c	c	c	h	—	h	a[c]
	III	c	c	a(c)	—	c	c	c	h	c	h	a
	IV	c[c]	c[c]	c[a]	—	c	c	c	h	c	h	c[a]
	V	c	c	c	—	c	c	c	h	c	h	c
	VI	c	c	a	—	c	c	c	h	c	h	c
	VII	c	c	a	—	c	c	c	h	c	h	c
	VIII	(e)c	c	a	—	c	h[1]	d	h	c	h	a
T. S. Just Below Cotyledons	I	e[c]	e[c]	c[c]	—	c	c	c	h	—	h	c[c]
	II	a	a	a[c]	—	c	c	c	h	c	h	a
	III	c	c(a)	c[a]	—	c	c	c	h	c	h	c(a)
	IV	e[a]	e[a]	c[a]	—	c	c	c	h	c	h	c[a]
	V	c	c	c	—	c	c	c	h	c	h	c
	VI	c	c	c	—	e	c	e	h	c	h	a
	VII	c	c	c	—	e	c	c	h	c	h	a[c]
	VIII	—	—	—	—	e	e	c	h	c	h	c

1) walls.

CAMBIUM. Although sometimes of pH 5·6 in the earlier stages, the cambium in the later stages has its reaction in the lower range, pH 5·2—4·8, which is common for the tissues lying between it and the relatively alkaline cortex and epidermis of the mature stem.

XYLEM. As elsewhere (see Chapter X—XII) the lignified walls of the xylem are at pH 4·4—4·0 from the beginning of lignification, but the xylem parenchyma is similar to the cambium in its reaction. In the mature plant near the base of the stem a few xylem fibres with very acid (k) walls were found.

PITH. More acid (e or c) in the seedling stage, the pith soon reaches a reaction of approximately pH 5·9 and remains in that range until maturity, except in the stem below ground where it varies to pH 5·6. In the mature plant, especially in the middle region of the stem, an outer more acid (lignified) layer can be differentiated from the relatively less acid central pith.

TISSUES OF THE ROOT. (Table V)

In general, the tissues of the root seem to have a more stable hydrion concentration than some of the stem tissues.

RADICLE. The tissues here are generally of pH 5·2—4·8, but the xylem walls in all stages, the dermatogen in the earliest stage of germination, and sometimes also the root-cap show a higher acidity, pH 4·4—4·0 (Tables I and II).

ROOT.

PILIFEROUS LAYER. This tissue is usually of pH 5·2—4·8, but occasionally pH 5·6. In certain stages of the old lateral roots a lesser acidity, pH 5·9, was observed. Any differences shown by the white as compared with the green variety are variations towards lesser acidity.

EXODERMIS and CORTEX. At all or practically all stages the reaction of the se tissues is similar to that of the piliferous layer, pH 5·2—4·8.

ENDODERMIS. In the main root the endodermis is uniformly of pH 5·2—4·8, but some variations occur in the lateral roots; both c and a have been observed sporadically as the reaction ranges of the cell-contents in these rootlets.

Table V

ROOT Stages		Piliferous Layer	Exodermis	Cortex	Endodermis	Pericyle	Phloem	Sieve-Tubes	Xylem	Pith
T. S. 9 cms. From Tip	I	c	c	c	e	e	e[ee]	- -	h	e(e)
	II	e	e	(a)	e	e	e	h	h	e[ee]
	III	e(a)	c(a)	ac[e]	e	e	e	h	h	ae[e]
	IV	c[e]	c[e]	e[de]	e	e	e	h	h	e[d]
	V	e	c	c	e	e	e	h	h	a
	VI	—	—	—	e	e	e	h	h	e
	VII	—	—	—	e	e	e	h	h	c
	VIII	—	—	e	e	e	e	h	h	a
T. S. Near Tip	II	c	e	c[ca]	e	c	e	- -	h	a(e)
	III	c[d]	c[c]	e[e]	e	e	e	—-	h	e
	IV	e[d]	c[e]	e[d]	e	e	e	- -	h	e[d]
	V	c	c	c	e	e	e	h	h	a[d]
	VI	—	—	—	e	e	e	h	h	a
	VII	—	—	—	e	e	e	h	h	a
	VIII	—	—	c	e	e	e	h	h	a
T.S. Young Lateral Root	II	c	a	a	e	e	e	- - -	h	e
	III	d[1]	a	a	ad[1]	—	d[1]	—	h	a
	IV	d[1]	ed[1]	ed[1]	e	e	ed[1]	- -	h	ed
	V	c	e	c	e[e]	—	e	-	h	
T.S. Old T.S. Root	II	e(c)	a	a	e	e	e(e)		h	ee
	III	a	a	a	e	e	e[d]	-	h	e[d]
	IV	e[d]	e[c]	e[d]	e	e	e[d]		h	e[d]
	V	c	c	c	e	e	e		h	e

PERICYCLE. This tissue, practically throughout, has the common range, pH 5·2—4·8.

PHLOEM. The sieve-tubes ,in all the later stages, show a relatively high acidity, pH 4·4—4·0. The phloem parenchyma is less acid, pH 5·2—4·8.

XYLEM. The walls of the vessels throughout are at pH 4·4—4·0 as usual, but the parenchyma is similar in reaction to the pericycle or pith.

---

[1] walls.

PITH. The central parenchymatous tissue of the root, although sometimes rather acid, pH 5·2—4·8 in the very young stages, shows a general decrease in acidity to pH 5·6 or pH 5·9 at all but the early stages. The region near the tip tends to be more regularly about pH 5·9, while higher up the main root and in the middle region of the lateral roots the reaction varies to pH 5·6 or even lower to pH 5·2—4·8. Where the white variety differs it is usually more acid.

TISSUES OF THE LEAF. (Table VI)

UPPER EPIDERMIS. A variation in the same specimen from pH 5·6 to pH 4·8 is common but the reaction rarely varies beyond that range. An exception occurs in the apex of the apical leaflet where, in the mature plant, all the tissues are around pH 5·9.

LOWER EPIDERMIS. In the immature plants of the green variety the lower epidermis is similar to the upper epidermis, but in the white variety the reaction reaches pH 5·9 at sporadic stages, and also normally in the mature plant of both varieties in the apex of the basal leaflet and in both base and apex of the apical leaflet.

GUARD CELLS. As far as it could be determined, the reaction of the guard cells of the stomata did not differ markedly from that of the neighbouring epidermal cells.

GLANDULAR HAIRS. These occur on the lower surface of the lamina and on the petiole. On the lamina the reaction of such hairs is usually pH 5·2—4·8, but on the petioles they are more acid, pH 4·4—4·0.

VASCULAR STRANDS. The xylem was again pH 4·4—4·0 at all stages. The phloem at the base of the leaf was more or less consistently of pH 5·2—4·8 in both varieties, but the reaction varied in the mature stage to pH 5·6 at the base of the basal leaflet and to pH 5·9 at the apex of the basal leaflet and at both base and apex of the apical leaflet.

CHLORENCHYMA. The palisade and spongy assimilating tissues were so similar that they can be considered together. The general reaction was around pH 5·9, and this was nearly constant in the lamina of the mature plant. The chlorenchyma of the petiole and of the stipules at the mature stage showed slightly greater acidity, pH 5·6. The chlorenchyma in the younger stages was not so constant in reaction, varying from cell to cell in the

Table VI

LEAF		Upper epidermis	Palisade Tissue	Spongy Tissue	Xylem	Phloem	Guard Cells	Lower epidermis	Hairs	
T. S. Near Apex	I	e(c)	a	a	—	c	e(c)	e(c)	e	
	II	ec[c]	a	a	h	c	c	c	—	
	III	e[ec]	a	a	h	d[1]	c	c	e	[1] walls
	IV	c	e[c]	e[c]	h	e[c]	e[c]	e[c]	e	
	V	ed[1]	c	c	h	ed[1]	e	c	e	[1] walls
	VI	e	a	a	h	e	e	e	h	
	VIII	a	a	a	—	a	a	a	—	Apex of Apical Leafl.
		c	a	a	—	a	a	a	—	Apex of Basal Leafl.
T. S. Near Base	I	c	e[ca]	a[ea]	h	e	—	e	e	
	II	ec	ca	ca	h	c	e	ec	e	
	III	ec	a[c]	a	h	e[c]	c	c[ca]	e	
	IV	e[ec]	e[c]	e[c]	h	e	e	e[ea]	h	
	V	d	e	e	h	e	c	e[ec]	e	
	VI	c	a	a	h	e	e	e	—	
	VII	c	c	a	h	e	e	e	--	
	VIII	c	a	a	h	a	a	a	--	Base of Apical Leafl.
		c	a	a	h	c	c	e	-	Base of Basal Leafl.
Petioles of Leaves as Expanded T. S. Near Lamina	II	ec[e]	ca[ea]	ca[ea]	h	e	---	e	h	
	III	ec	a	a	h	c	—	ec	h	
	IV	e	e	e	h	e	--	e	h	
	V	ec	c[ea]	c[ea]	h	[ce]	—	c[e]	h	
	VI	c	a	a	h	c	—	c	h	
	VII	c	a	a	h	c	—	c	h	
	VIII	c	c	c	h	c	—	c	h	Apical Leaflet
		c	ca	ca	h	ah	—	e	h	Basal Leaflet
T. S. Base of Stipule	I	e	e	e	h	e	—	e	—	
	II	ec	ec[ea]	e[ea]	h	e	—	e	—	
	III	c	c	c	h	c	—	c	—	
	IV	e	e[c]	e[c]	h	e	—	c	—	
	V	e	e[ec]	e[ec]	h	e	—	c	—	
	VI	e	c	c	h	c	—	e	—	
	VII	e	c	c	h	c	—	e	--	

same specimen and from stage to stage but keeping within the range pH 5·9 to pH 4·8. The reaction at the apex of the lamina was more regularly at pH 5·9 than it was at the base of the lamina or in the petiole, while the stipular chlorenchyma very seldom rose above pH 5·6.

LEAVES FROM DIFFERENT LEVELS. On account of the natural green colour there is considerable difficulty in judging the colours given by the indicators in chlorenchymatous tissues, so that all the above figures are given with some reserve. At the same time it was found possible to get some indication of a variation in the reaction of leaves from different parts of the stem. Our observations support the conclusion that the actual acidity decreases as the stem is ascended, thus pH 5·2—4·8 appears to be common in the chlorenchyma of the lower leaves, while pH 5·9 occurs in that of the fifth and sixth leaves up the stem. As this distribution of the actual acidity is exactly the reverse of that obtained by ASTRUC (1903) for the titrimetric acidity due to "free and half-combined" acids in the expressed juice of *Phaseolus*, a careful re-investigation of this point is required before the conclusion can be accepted. The distribution of acids was, however, found by that author to vary in other cases from morning to evening; and further, it is quite possible for the actual acidity of the unbroken cell contents to vary inversely with the titrimetric acidity of the expressed juice when the buffer conditions are also varying, as seems to be probable from the data given by ASTRUC. The reverse variations found by GUSTAFSON (1924a) should also be kept in mind in this connection.

TISSUES OF THE FLOWER. (Table VII)

The reactions of these tissues were the same in both varieties, with the exception of one plant of the white variety which showed the following differences — **c** instead of **e** in the epidermis of the calyx and corolla, and **h** instead of **e** in the vascular tissue of the calyx.

The only interesting changes during the maturing of the flowers were the decrease in acidity in the anthers, pH 5·6 to pH 5·9, and in the pollen grains, pH 5·2—4·8 to pH 5·6. The mature pollen grains, as in the sunflower (p. 191), showed a higher pH (5·6) than that of the stigma (pH 5·2—4·8).

Table VII. *Flowers at all stages*

YOUNG PRIMORDIA, Glandular Hairs, Young Pollen — all e.
YOUNG STAMENS — Pollen e, Anther e, Filament e.
OLD „ — Pollen e, Anther a, Connective e, Filament ec, Conducting Strands e, Tube Region a, Epidermal Walls d.
YOUNG CARPEL — Style, Stigma, Glandular Hairs — all e, Ovary Wall e, Conducting Strands h, Ovules e.
OLD CARPEL — Style, Stigma, Hairs — e.
YOUNG POD, 5 cms. long — Vascular tissue h, All other tissues e.
T. S. ditto — Vascular tissue h. Other tissues e.

COROLLA of Mature Flower. . . .	Base	Apex
Upper epidermis	e[e]	e[e]
Mesophyll	d	d[e]
Vascular tissue	h	h
Lower epidermis	e[e]	e[e]
CALYX of Mature Flower (Base)		
Epidermis	e[e]	
Mesophyll	d[e]	
Vascular tissue	e[h]	

CONCLUSIONS

General phenomena bearing upon the factors which may influence the reaction of tissues during the growth of a plant have been discussed in connection with the previous investigation of the tissue reactions of *Helianthus annuus* (p. 201). There is also available a useful discussion mainly on the zoological side by REISS (1926).

We do not propose at this stage to make a detailed comparison of the tissue reactions of *Vicia faba* and *Helianthus annuus*, rather would we emphasise firstly that there are differences and similarities, and secondly that detailed data of tissue reactions are as yet available only from these two species. Altogether it seems to us that it would be wiser not to discuss the significance of such differences as are found until more data concerning the buffers of both forms are obtained. These are at the moment being investigated in this laboratory, with interesting results.

It may, however, be convenient to summarise the more obvious points of contact between the results of the present investigation and the above-mentioned tissue-reactions of the sunflower.

BEAN and SUNFLOWER. The cortex is usually of a somewhat similar reaction in the two species, but in the bean there is no markedly acid epidermis such as is present in the sunflower. The stelar tissues in the stem show a general similarity, except in the endodermis and pericycle which are less acid in the sunflower stem. In the root the cortex, endodermis, pericycle and pith are less acid in the case of the bean than in the sunflower The increase in acidity, which was observed for several tissues of the sunflower in passing from the aerial to the subterranean parts of the axis, does not appear in the broad bean.

The reaction of the chlorenchyma of the leaf has been estimated, with reserve, as being slightly less acid in the bean than in the sunflower. In both plants there is a somewhat indefinite tendency for the lower epidermis of the leaf to be less acid than the upper epidermis. This phenomenon seems to be related to the part of the leaf or leaflet examined and a detailed investigation is required.

In both species the mature pollen grains are less acid than the mature stigma.

These similarities and differences may be related to buffer conditions rather than to any general metabolic conditions. It is yet too early to suggest any causes for the phenomena which are being recorded.

SUMMARY

The Range Indicator Method has been applied to a detailed study of the tissue reactions of the broad bean, *Vicia faba*, at various stages of development and throughout the seed, seedling and mature plant. The results are recorded, summarised and briefly compared with those obtained for the sunflower, *Helianthus annuus*.

2. THE BUFFERS OF BEAN STEM AND ROOT

The data are presented below almost exactly as given by Miss S. H. Martin (1927) because the buffer index point of view is considered in the last section of this chapter and it is desired here to maintain the historical sequence in the development of the work.

INTRODUCTION

The relation of pH to the physiology of plant and animal life has been dealt with in various treatises, where the importance of buffer action in controlling the normal life processes has been emphasised especially for animal fluids. More recently similar considerations have been applied to the sap of plant cells, where it has been found that certain substances are present which act in the same manner as do the regulators of the normal reactions of animal tissues.

Each buffer system has its own fixed range which depends on the nature of the constituents, while the amount of buffer action depends not only on the nature of the constituents but on the relative concentrations of the reacting substances.

Where two or more buffer systems occur together there will be complex interactions between the systems and these systems together will maintain the pH at what we may call its normal value.

In succulent plants organic acids are formed, sometimes in considerable quantities, and the amounts formed depend on several factors such as light, amount of carbohydrate present, &c., and as the buffer value of the sap is dependent on the amount of acid and salts of acids present we see how external factors may indirectly affect the buffer value of the sap.

In a few non-succulents buffer elements have also been shown to be effective within the normal pH zones of the plant tissues (Chapter XII).

The results of HAAS' experiments (1920) with the expressed sap of Buckwheat and Clover suggest that some buffer substance or substances were active in controlling the reactions of these plant saps within the pH regions in which the titrations were carried out.

The pH values of the tissues of Broad Bean have recently been recorded. These observations show a certain degree of variation which at times seems to be rather spasmodic, indicating that the buffer conditions in the cell-sap of the tissues of this plant may vary to some extent.

These changes in reaction of the sap of the tissues of Bean may be due to the presence of varying amounts or to the absence of certain chemical substances which are capable of exerting buffer action within their respective ranges.

Supposing we have present in a plant cell some substances capable of maintaining the reaction of the sap at a certain fixed point and, owing to metabolic activities within the cell, other substances are formed which can exert a buffer action within a different range either on the acid or on the alkaline side of the former pH range. Where these two ranges overlap there will be interactions between the systems which will tend to balance one another and the pH will assume an intermediate value, i. e. an equilibrium will be formed and as the relative amounts of the reacting substances of one or other of the opposed systems increase or decrease a new equilibrium will be formed and the pH value will be urged either towards the acid side or towards the alkaline side according to which system predominates.

A number of standard buffer solutions are used for physiological purposes. Some of these are mixtures of salts of several organic acids which interact in such a way that a continuous buffer range, within wide limits, is obtained. ACREE, MELLON, AVERY and SLAGLE describe a stable single buffer solution which is effective between pH 1 and pH 12 (1921). At any point in this wide range one or other of the reacting systems predominates and controls the reaction, and any change in the proportions of the constituents of the solution produces a corresponding change in pH.

The following account gives a description of the experiments carried out in order to ascertain something of the buffer conditions within the expressed sap of Broad Bean, stem and root.

Variations in the actual amounts of the buffer substances present do occur. The possible significance of these variations will be referred to later.

PHOSPHATE BUFFER VALUES

Using the same methods as have been described in the case of Sunflower (p. 212) the expressed saps of Bean stem and root were examined for phosphate content and buffer capacity. All buffer values are given in terms of molar phosphoric acid. Owing to the darkening of the expressed sap all pH and buffer determinations were carried out immediately after expressing the sap.

The results of these analyses are given in tabular form.

BEAN STEM

Table VIII

pH of sap	c. c. sap	c.c.NaOH added	pH after add. of alk.	Buffer value	H_3PO_4 content	Diff. between buffer value and H_3PO_4 content
5·8	2	1·0	6·4	0·01412	0·0069	0·0072
5·8	2	1·6	6·6	0·01423	0·0069	0·0073
5·8	2	2·0	6·8—6·6	—	0·0069	—
5·8	2	2·4	6·8	0·0151	0·0069	0·0082

Table IX

5·8	2	0·5	6·2	0·0128	0·0126	0·0002
5·8	2	1·0	6·4	0·0141	0·0126	0·0015
5·8	2	1·5	6·6	0·0130	0·0126	0·0004
5·8	2	2·0	6·8—6·6	—	0·0126	—
5·8	2	2·5	6·8	0·0157	0·0126	0·0031

Table X

5·8	2	0·3	6·0	0·0192	0·0134	0·0058
5·8	2	0·8	6·2	0·0206	0·0134	0·0072
5·8	2	1·2	6·4	0·0198	0·0134	0·0064
5·8	2	2·4	6·6	0·0213	0·0134	0·0079
5·8	2	3·4	6·8	0·0214	0·0134	0·0080
5·8	1	0·4	6·2	0·0206	0·0134	0·0072
5·8	1	0·15	6·0	0·0192	0·0134	0·0058
5·8	1	0·7	6·4	0·0198	0·0134	0·0064
5·8	1	1·2	6·6	0·0213	0·0134	0·0079
5·8	1	1·7	6·8	0·0214	0·0134	0·0080

Table XI

5·8	2	0.25	6·0	0.0160	0.0108	0·0052
5·8	2	0·65	6·2	0·0176	0·0108	0·0059
5·8	2	0·8	6·4—6·2	—	0·0108	—
5·8	1	0·8	6·6—6·4	—	0·0108	—
5·8	1	0·9	6·6—6·4	—	0.0108	—
5·8	1	1·0	6·6	0·0178	0·0108	0·0070

Table XII

pH of sap	c. c. sap	c. c. NaOH added	pH after add. of alk.	Buffer value	H_3PO_4 content	Diff. between buffer value and H_3PO_4 content
5·6	2	0·25	5·8	0·0162	0·0128	0·0034
5·6	2	0·5	6·0	0·0161	0·0128	0·0033
5·6	2	1·0	6·4—6·2	—	0·0128	—
5·6	2	1·2	6·4—6·2	—	0·0128	—
5·6	2	1·6	6·4	0·0185	0·0128	0·0057
5·6	1	0·8	6·4	0·0185	0·0128	0·0057
5·6	1	1·6	6·8—6·6	—	0·0128	—
5·6	1	1·8	6·8	0·0206	0·0128	0·0078

Table XIII

6·0	1	0·4	6·4—6·2	—	0·0118	—
6·0	1	0·5	6·4	0·0181	0·0118	0·0063
6·0	1	0·9	6·6	0·0189	0·0118	0·0071

Table XIV

6·0	1	0·2	6·2	0·0172	0·0107	0·0065
6·0	1	0·4	6·4—6·2	—	0·0107	—
6·0	1	0·5	6·4	0·0181	0·0107	0·0074

Table XV

6·0	1	0·4	6·4	0·0145	0·0095	0·0050
6·0	1	0·5	6·6—6·4	—	0·0095	—
6·0	1	0·8	6·6	0·0161	0·0095	0·0066
6·0	1	1·0	6·8—6·6	—	0·0095	—
6·0	1	1·2	6·8	0·0168	0·0095	0·0073

Examination of Table VIII shows that some buffer substance or substances other than the inorganic phosphates were present in the sap. These substances were capable of buffering the sap, between pH 5·8 and pH 6·4, to the extent of a 0·0072 M H_3PO_4 solution, and between pH 5·8 and pH 6·8 to the extent of a 0·0082 M H_3PO_4 solution; also within the reaction zone pH 6·4—pH 6·8 there was an increased buffer value equal to that of a 0·0010 M phosphoric acid solution.

Table IX shows that between pH 5·8 and pH 6·4 the buffer value was higher than the inorganic phosphate content, the difference between buffer capacity and the actual phosphate present being equal to a 0·0015 M phosphate solution. Between pH 5·8 and pH 6·8 there was a difference in buffer value corresponding to a 0·0031 M phosphate solution. The increase between pH 6·4 and pH 6·8, therefore, corresponded to a 0·0016 M phosphate solution.

From Table X we see that between pH 5·8 and pH 6·4 the expressed sap showed a buffer capacity which was slightly more than 50 per cent. greater than that due to the amount of inorganic phosphate present. Between pH 6·0 and pH 6·4 we get an increase of 0·0006 M, while between pH 6·4 and pH 6·8 there was a further increase from 0·0198 M to 0·0214 M giving a difference between pH 6·4 and pH 6·8 corresponding to that of a 0·0016 M phosphoric acid solution.

Table XI. Between pH 5·8 and pH 6·0 the difference between buffer value and H_3PO_4 content equalled a 0·0052 M phosphate solution; between pH 5·8 and pH 6·2 the difference was 0·0059 M. Between pH 5·8 and pH 6·6 the sap was buffered by substances other than the inorganic phosphates present, to the extent of a 0·0070 M phosphoric acid solution. Between pH 6·0 and pH 6·6 there was an increased buffer value corresponding to that of a 0·0018 M phosphoric acid solution.

Similar considerations apply to Tables XII, XIII, XIV and XV.

The above tabulated results of experiments with the expressed sap of Bean stem show that, at all the reaction points determined, the buffer capacity of the sap was higher than that due to the amounts of inorganic phosphates present. The differences between buffer value and inorganic phosphate content varied from sample to sample of the sap.

The reaction of the expressed sap in most cases was pH 5·8; pH 5·6 was observed in one case and pH 6·0 in a few cases.

The experiments were carried out to an arbitrary end-point pH 6·8 in most cases.

In all cases, on titration with standard alkali, the sap exhibited a progressive increase in buffer value, with increasing amounts of alkali added, from the original pH of the expressed sap to reaction points in the direction of lesser acidity.

The increase of buffer value within the reaction points determined also varied from sample to sample as will be seen from the following figures taken from the tables above.

BEAN STEM

Table XVI

pH sap	Buffer value between 5·8—6·4	Buffer zone	Increase of buffer value	H_3PO_4 content of sap
5·8	·0141	6·4—6·8	0·0010 M	·0069 M
5·8	·0141	6·4—6·8	0·0016 M	·0126 M
5·8	·0198	6·4—6·8	0·0016 M	·0134 M
5·6	·0191	6·4—6·8	0·0021 M	·0128 M

	Buffer value between 6·0—6·4			
6·0	·0145	6·4—6·8	0·0023 M	·0095 M
6·0	·0181	6·4—6·6	0·0008 M	·0118 M
6·0	·0181	6·2—6·4	0·0009 M	·0107 M
5·8	—	6·0—6·6	0·0018 M	·0108 M
		6·2—6·6	0·0011 M	·0108 M

From this table we see that the buffer value of the expressed sap between pH 5·8 and pH 6·4 varied from 0·0141 M to 0·0198 M, and between pH 6·0 and pH 6·4 from 0·0145 M to 0·0181 M.

The increase in buffer capacity from pH 6·4 to pH 6·8 varied from 0·0010 M to 0·0023 M.

The actual amounts of inorganic phosphates present in the sap varied from 0·0069 M to 0·0134 M.

BEAN ROOT

In the root the inorganic phosphate content varied from 0·0079 M to 0·0092 M.

The reaction of the expressed sap was sometimes at pH 5·6, sometimes at pH 5·8 and more often at pH 6·0.

Owing to the necessary washing of the roots and the resulting dilution of the sap, buffer determinations and phosphate analyses cannot be directly compared with those of the stem.

The results of four experiments with the expressed sap of Bean root are given below.

BEAN ROOT

Table XVII

pH of sap	c.c. sap	c.c. NaOH added	pH after add. of alk.	Buffer value	H_3PO_4 content	Diff. between buffer value and H_3PO_4 content
5·8	2	0·2	6·0	0·0128	0·0092	0·0036
5·8	2	0·5	6·2	0·0129	0·0092	0·0037
5·8	1	0·5	6·4	0·0141	0·0092	0·0049

Table XVIII

6·0	1	0·3	6·4	0·0109	0·0082	0·0027
6·0	2	0·2	6·2	0·0086	0·0082	0·0004
6·0	2	0·5	6·6—6·4	—	0·0082	- -
6·0	2	0·6	6·6—6·4	—	0·0082	- -
6·0	2	1·2	6·6	0·0124	0·0082	0·0042

Table XIX

5·6	2	0·4	6·0	0·0103	0·0079	0·0024
5·6	2	0·5	6·2—6·0	—	0·0079	-
5·6	2	0·8	6·2	0·0147	0·0079	0·0068

Table XX

6·0	2	0·2	6·2	0·0086	0·0088	0·0002
6·0	1	0·3	6·4	0·0109	0·0088	0·0021
6·0	1	0·5	6·6 -6·4	- -	0·0088	
6·0	1	1·0	6·8	0·0137	0·0088	0·0049
6·0	1	1·5	7·0	0·0157	0·0088	0·0069

Table XVII. The sap in this case had a buffer value greater than that due to the inorganic phosphates present. Between pH 5·8 and pH 6·4 a buffer value corresponding to that of a 0·0049 M phosphate solution was in excess of that due to the contained phosphate. Within the range pH 6·0 — pH 6·4 there was an increased buffer capacity equivalent to that of a 0·0013 M phosphate solution.

Table XVIII. The reaction of this sap was pH 6·0.

The addition of 0·2 c. c. NaOH 0·005 M shifted the reaction to pH 6·2 indicating a buffer value corresponding to that of a 0·0086 M phosphate solution.

The phosphate content was 0·0082 M so that the contained inorganic phosphates accounted for somewhat less than the total buffer capacity, to the extent of a 0·0004 M solution.

Between pH 6·2 and pH 6·6 there was an increased buffer value from 0·0086 M to 0·0124 M, i. e. 0·0038 M. Between pH 6·0 and pH 6·6 there was a buffer value greater by 0·0042 M, than the amount of inorganic phosphates present.

Tables XIX—XX show similar results.

In the root as in the stem the buffer capacity of the expressed sap was higher than that of the contained inorganic phosphate. The buffer value showed progressive increase as the reaction was shifted, by the addition of increasing amounts of alkali, in the direction of lesser acidity, i. e. *towards pH 7·0.*

All the records given above show how necessary it is to state the range in which the buffer capacity is determined, as within different reaction points we may get a totally different value.

IDENTIFICATION OF OTHER BUFFERS

TESTS FOR MALIC ACID. — Preliminary qualitative tests for organic acids indicated the presence of malates in the sap of Bean.

A quantity of sap was acidified with HCl in dilute solution, and was then extracted with ether. To the ether extract neutralised with NaOH, excess $CaCl_2$ conc. was added and the resulting precipitate was filtered off. This filtrate was boiled and when $CaCl_2$ was again added there was no precipitate. To this filtrate hot absolute alcohol was added when traces of a precipitate were found. On boiling the alcoholic solution a white precipitate of *calcium malate* was obtained.

To test this precipitate, the calcium malate was dissolved in hot water and on addition of lead acetate a white precipitate was found. This precipitate was suspended in water and decomposed by bubbling sulphuretted hydrogen through the solution. The lead sulphide was then filtered off and the solution concentrated on a water-bath.

The concentrated solution was then tested for malic acid with β-naphthol-sulphuric acid and gave the characteristic colour reactions i. e. (1) greenish yellow colour, (2) bright yellow on heating, (3) bright orange solution on adding a little water.

Measured quantities of sap were then analysed for malic acid content.

Experiment I. Using 45 c. c. sap, the weight of calcium malate obtained was ·08 grammes, which is equal to 1·777 grammes per litre. Therefore, the malic acid content was 1·384 grammes per litre of sap.

Experiment II. Using 30 c. c. sap, the calcium malate obtained was 1·733 grammes per litre which is equivalent to 1·352 grammes of malic acid per litre.

Experiment III. Using 60 c. c. sap the weight of calcium malate determined was 2·125 grammes per litre. The malic acid content was, therefore, 1·655 grammes per litre.

Experiment IV. Using 20 c. c. sap the calcium malate precipitate weighed ·035 grammes. This is equal to 1·75 grammes of calcium malate per litre; therefore the malic acid content was 1·363 grammes per litre of sap.

A malic acid solution containing 1·6 grammes of malic acid per litre was prepared. This solution was acid to Methyl Red and was brought to pH 5·8 by the addition to 10 c. c. of solution of 0·3 c. c. N. NaOH.

The solution, now containing 1·553 grammes malic acid per litre, was tested for buffer value between the reaction points pH 5·8 and pH 6·0.

The addition of 0·2 c. c. NaOH 0·005 M shifted the reaction from pH 5·8 to pH 6·0; therefore, the buffer value of the malate solution in this narrow zone corresponded to that of a 0·00256 molar phosphate solution.

As pH 6·0—pH 5·8 is near the unbuffered zone, on the alkaline side, for a malate buffer system, the degree of buffer action of such quantities of malic acid at more acid points will be considerably greater.

To test the buffer value of Bean Sap on the acid side of its normal reaction, standard acid (0·005 M hydrochloric) was added. The addition of 0·3 c. c. HCl brought the reaction to pH 5·4 while 0·4 c. c. brought the reaction to pH 5·2.

This shift in reaction would be caused by the addition of an equal amount of acid to a 0·0142 molar phosphoric acid solution, and this gives the phosphate buffer value of the sap between the reaction points pH 5·8 and pH 5·2.

Another portion of this sap sample gave a buffer value of 0·0127 M between pH 5·8 and pH 6·0 and 0·0128 M between pH 5·8 and pH 6·2, indicating a higher buffer value on the acid side than on the alkaline side. This higher buffer value on the acid side of the normal reaction of the expressed sap will be accounted for, in part at least, by the presence of malates in the sap. Other organic acids if present may contribute to the higher buffer value on the acid side of the normal reaction. Tests for other organic acids were carried out but with the exception of oxalic acid the presence of any of these was not detected.

TESTS FOR OXALIC ACID. — A large quantity of expressed sap of Bean stem was acidified with acetic acid and the following experiments were carried out.

Experiment I. The addition of calcium acetate to a portion of the acidified sap caused a white precipitate of needle crystals of calcium oxalate mixed with characteristic crystals of calcium sulphate. After the addition of barium chloride (ZIMMERMANN 1893) the calcium oxalate crystals remained unchanged and the calcium sulphate crystals became coated with a granular precipitate and finally broke up.

Experiment II. The addition of lead acetate to another portion of acidified sap gave a white precipitate.

Experiment III. A portion of sap, acidified with acetic acid was extracted with ether. The ether layer, containing the organic acids in solution was separated off from the insoluble substances. After evaporating off the ether the solution was neutralised with NaOH. The addition of calcium chloride (5 per cent. solution) to the neutral solution caused a white powdery precipitate which was insoluble in acetic acid and soluble in hydrochloric acid.

In order to confirm the presence of oxalate, calcium acetate was added to a sample of sap acidified with acetic acid. The resultant precipitate was filtered off and dissolved in HCl and was then extracted with ether. On evaporating off the ether white crystals of calcium oxalate separated out. These were in the form of crystalline masses which were soluble in HCl, soluble in HNO_3, insoluble in KOH and insoluble in acetic acid.

The calcium oxalate crystals obtained from a 45 c. c. sample of sap were filtered off and washed with water. The crystals were then dissolved; H_2SO_4 was added and the solution was titrated at 60° C with an $\frac{N}{10}$ permanganate solution. Result: — 1·9 grammes oxalic acid per litre of sap were estimated, i. e. oxalates equivalent to a 0·0211 M oxalic acid solution were present in the expressed sap.

Portions of expressed sap were tested for other organic acids e. g. tartaric, citric, succinic etc., but the presence of any of these was not detected.

OTHER BUFFERS. — An oxalate buffer system is capable of stabilising the reaction of a solution within the range pH 3·0 to pH 5·3.

Malates are effective between pH 3·7 and pH 6·0. These two systems together with the phosphates present form a mechanism capable of buffering within a wide range but beyond pH 6·0 in the direction of lesser acidity the presence of substances other than the inorganic phosphates is necessary to account for the higher buffer value found by titration with alkali.

Of the possible known systems which may supplement the buffer value of the inorganic phosphates we have proteins and bicarbonates.

PROTEIN AND AMINO-ACIDS. — The part played by proteins has been dealt with by various authors including Reiss and Loeb. Reiss (1926) attaches considerable importance to the role of proteins and their hydrolytic products. According to this author "Les substances protéiques et leur produits de dégradation réalisent les effets tampons les plus considérables".

Reiss bases his considerations mainly on observations of animal tissues. In the cell sap of plants proteins and amino-acids do not occur in such considerable quantities as in animal fluids, e. g. muscle juice, and in consequence not much importance has been attached to them so far as their value as buffers is concerned.

According to a recent view (p. 74) amino-acids are of much more importance biologically than has hitherto been realised. The 'Zwitterions', carrying positive and negative charges, are considered to be present, in amino-acid solutions, in appreciable quantities, and as ampholytes these Zwitterions if present in quantity may be capable of exerting a quite considerable buffer effect.

The acid and basic dissociation constants for several amino-acids are given by BJERRUM (see CLARK 1928). These are considerably higher than those given before, which means that proteins and amino-acids in a medium more acid or less acid than their iso-electric points may exhibit much stronger basic or acidic properties than has usually been attributed to them.

For this reason these amphoteric substances may be responsible for some of the buffer capacity of the expressed sap of Bean, within the reaction points determined, on the alkaline side of pH 6·0.

There is present in all parts of Broad Bean an amino-acid (dihydroxyphenylalanine) which, as it readily oxidises in air, is held to be responsible for the purplish black colour which occurs when the tissues are exposed to air.

An attempt was made to discover the part played by any proteins present in the sap, by precipitating the proteins by heating and comparing the buffer value of the juice before and after removal of the proteins. The addition of trichloracetic acid to the cooled and filtered juice caused no precipitate showing that all the proteins had been removed by heating.

The following comparison of the results from unheated, and heated and cooled juice are given with reserve, as the effects of heating the sap are complex, and may result in complete disturbance of the normal sap conditions.

Among the possible effects of such heating are precipitation, along with the proteins, of traces of inorganic phosphates and other substances, driving off of CO_2 from carbonates and bicarbonates, oxidation of various substances, the formation of substances more or less highly dissociated, physical effects associated with surface tension changes, and so on; all of which changes may alter the pH and may influence the buffer value of the extract.

Details of two experiments with the sap of Bean stem and one with the sap of Bean root, are given below.

Experiment I. The expressed sap (unheated) had a reaction value of pH 6·0. The buffer value of this sap between pH 6·0 and pH 6·4 equalled that of 0·0181 molar phosphoric acid solution; between pH 6·0 and pH 6·6 that of a 0·0189 molar phosphoric acid solution. The inorganic phosphate content was 0·0118 M.

The filtered extract after heating and cooling, gave a pH value of 6·0 and the buffer capacity of this juice between pH 6·0 and pH 6·4 was 0·0181 M, and between pH 6·0 and pH 6·6 was 0·0189 M, so that neither the reaction value nor the buffer capacity of the juice in this particular case had been altered by heating. The inorganic phosphate content of the cooled extract was 0·0121 M, i. e. 0·0003 M more than was obtained from the unheated sap, so that the possible protein buffer value here may have amounted to that of a 0·0003 molar phosphoric acid solution.

Experiments II and III are given in tabular form.

Experiment II. Table XXI

BEAN STEM — SAP UNBOILED

pH of sap	c. c. sap	c. c. NaOH added	pH after add. of alkali	Buffer value	H_3PO_4 content
6·0	1	0·4	6·4	0·0145	0·0095
6·0	1	0·8	6·6	0·0161	0·0095
6·0	1	1·2	6·8	0·0168	0·0095

BEAN STEM — SAP BOILED

pH of sap	c. c. sap	c. c. NaOH added	pH after add. of alkali	Buffer value	H_3PO_4 content
6·2	1	0·2	6·4	0·0125	0·0088
6·2	1	0·5	6·6	0·0136	0·0088
6·2	1	1·0	6·8	0·0166	0·0088
6·2	1	1·5	7·0	0·0178	0·0088

This table shows that the addition of 0·8 c. c. alkali to the expressed sap and to the protein free extract, both brought to pH 6·4, shifted the reaction of each from pH 6·4 to pH 6·8, indicating equal buffer value in both cases, between these points.

The phosphate content of the filtered extract was lower than that of the unheated sap and the reaction was less acid.

It is clear, from this experiment, that some disturbance of the normal conditions had taken place. The CO_2 dissolved in the liquid as H_2CO_3 or present in the form of carbonates and bicarbonates may have been driven off by heating, thus rendering the juice less acid; while the formation of acid groups, probably derived from the proteins and capable of buffering in this region may have brought the buffer value up to the level of that of the unboiled sap. Some of the inorganic phosphates may have been precipitated along with the proteins. Other chemical and physical effects may also have intervened.

These considerations show that although a casual comparison of the buffer values in the above tables, may lead one to believe that the buffer value of the protein free juice is lower than that of the expressed sap containing proteins, the real state of affairs may be quite otherwise.

Any comparison of buffer values must be of those within a definite fixed limit, since outside of this region the buffer values may be totally different. As the tables given above show, the buffer value between pH 6·0 and pH 6·8 does not correspond to that found between pH 6·2 and pH 6·8.

Experiment III. Table XXII

BEAN ROOT — SAP UNBOILED

pH of sap	c. c. sap	c. c. NaOH added	pH after add. of alkali	Buffer value	H_3PO_4 content
6·0	2	0·2	6·2	0·0086	0·0088
6·0	1	0·3	6·4	0·0109	0·0088
6·0	1	0·5	6·6—6·4	—	0·0088
6·0	1	1·0	6·8	0·0137	0·0088
6·0	1	1·5	7·0	0·0157	0·0088

BEAN ROOT — SAP BOILED

6·2	2	0·4	6·4	0·0125	0·0088
6·2	1	0·2	6·4	0·0125	0·0088
6·2	1	0·5	6·6	0·0136	0·0088
6·2	1	1·0	7·0 —6·8	—	0·0088
6·2	1	1 2	7·0	0·0143	0·0088

Comparison of the amounts of alkali required to produce a shift from pH 6·4 to pH 7·0 shows that the buffer value between these reaction points was higher in the case of the filtered extract than in that of the unboiled sap. The reaction of the boiled extract was less acid, but the phosphate content was unaltered.

Other experiments with unheated, and heated, cooled and filtered sap of stem and root, gave similar results to those tabulated above. The filtered extract sometimes had a higher buffer value, while the pH was usually slightly less acid or unaltered.

The possible results of heating the sap are too complex for us to draw any definite conclusions from the above experiments.

Within what pH regions, and to what extent plant proteins may contribute to the buffer value of the cell-sap, I do not venture to say; but in certain animal juices it is known that the colloidal proteins systems act as buffers within such reaction zones as have been determined for the expressed sap of Bean, and it may be that here too proteins, or their derivatives, may have played a part in buffering the sap, within those reaction zones.

The actual amount of the protein which was precipitated by heating, was not determined.

BICARBONATE-CARBONIC ACID SYSTEM. — Consideration of the reactions of some of the tissues of Bean, e. g. those of the cortex and pith, suggested that in these tissues carbonates, if present, might be responsible for some of the buffer action exhibited by the expressed sap, which is a mixture of saps from the various tissues and probably consists mainly of sap derived from cortex and pith.

A bicarbonate-carbonic acid system is capable of buffering on the alkaline side of pH 5·8. Beyond pH 5·8 in the direction of greater acidity, any carbonate present in the sap will exist as free acid, but beyond pH 5·8 in the direction of lesser acidity any carbonate present will form a bicarbonate-carbonic acid system, which will show feeble buffer activity at either end of its buffer zone, but will exhibit increasing buffer value as the reaction progresses towards the point of maximum buffer capacity for this system, at or near pH 7·0. If a bicarbonate-carbonic acid system is present in the expressed sap of Bean we should expect to find increasing buffer value as the titration of the sap proceeds in the direction of pH 7·0.

That this is indeed the case has already been pointed out. The expressed sap of Bean stem was tested for carbonate content. To a measured quantity of sap in a burette a known volume of a concentrated solution of tartaric acid was added from another burette. Bubbles of CO_2 were evolved. By adjusting the levels of the liquids in the two burettes the pressure of the gas to be analysed was brought to that of the atmosphere. The burette was now attached to a HALDANE's gas analysis apparatus and a sample of the gas was analysed for CO_2 content, by absorption of the CO_2 in potash solution. All readings were taken at room temperature. Varying amounts of sap (8. c. c.—10 c. c.) were used for the analyses.

The figures given are calculated as c. c. of carbon-dioxide per 100 c. c. of sap.

Amounts of CO_2 in c. c. calculated to 100 c. c. of sap were as follows: — 7·2, 4·0, 7·5, 7·3, 3·1, 7·3.

The amount of CO_2 present in the carbonate or bicarbonate form was not determined, so we cannot say to what extent a bicarbonate-carbonic acid system was present, but from the amounts of CO_2 driven off by the acid, it seems reasonable to conclude that such a buffer system was present in the expressed sap, and that this bicarbonate system contributed to the degree of buffer action exhibited by the expressed sap, on the alkaline side of pH 5·8.

All the results obtained by titration of the sap with alkali point to the presence of such a buffer system.

INTERACTION OF BUFFERS. — If we examine the buffer range of the substances, found in the expressed sap of Bean, which are known to be capable of exerting buffer action within their respective ranges we see how overlapping systems may interact to stabilise the pH of the sap within a wide region. Oxalates buffer between pH 3·0 and pH 5·3, malates are effective between pH 3·7 and pH 6·0. From pH 3·7 to pH 5·3 the pH value maintained by the interactions of both these systems will depend on the relative concentrations of the reacting substances. The buffer zone for inorganic phosphates is pH 5·2 to pH 8·0, so that malates and phosphates will interact to buffer the sap between pH 5·2 and pH 6·0. Where the inorganic phosphate system comes into action at pH 5·2 all or practically all the oxalic acid present will exist as inactive salt, e.g. calcium oxalate, crystals of which occur frequently in plant cells. Beyond pH 5·8 a bicarbonate system is effective, so that where malates cease to have any buffer value bicarbonates begin to exert their influence and the pH of the sap will be controlled by inorganic phosphates and bicarbonates.

Proteins and their derivatives, and possibly other substances, under proper conditions, may also play a part.

HAAS (1920) gives a record of one plant with sap on the alkaline side of pH 7·0. The juice from the tops of sweet clover had a reaction of approximately pH 7·3, and contained about

50 per cent. more CO_2 than that of medium white clover, the sap of which was of pH 6·0.

Consideration of the results of the titration of Bean sap and comparison of these results with those of other workers using various plants, seem to indicate that in plant cells with sap reactions normally on the alkaline side of pH 6·0 bicarbonate if present, may alone or in conjunction with other systems maintain constant the relatively low degree of acidity.

CARBON DIOXIDE EFFECTS. — All records of the pH of cell contents show that in very few plant cells is there a reaction value higher than pH 6·2, so that under normal conditions most plant cells are not buffered by a bicarbonate system which at pH 6·0 can exert only very feeble buffer action. At more acid points this system disappears and one of its products, i. e. CO_2, may not only produce shifts in reaction but in the living plant may enter into combination with other substances to form organic compounds, e. g. organic acids which are capable of rendering the sap more acid and of maintaining the acidity at the lower pH value.

As has already been pointed out variations in the pH of the cell contents of the tissues of Bean "from plant to plant and from stage to stage of the growth" do occur (p. 233). These variations may be accounted for by the presence or absence of one or more buffer substances, e. g. oxalates, malates, inorganic phosphates, bicarbonates &c., which occur in variable amounts in the expressed sap and presumably also vary in amount in the individual cells of this plant.

The disadvantages of using expressed sap have been pointed out in Part II. It is possible and indeed extremely probable that substances not in contact with each other in the living cell, may be brought into contact during the extraction of the sap.

Comparison of the buffer conditions within the cell sap of each tissue of the Bean would be an interesting study, if it were possible to obtain sap from each tissue separately, and in sufficient quantity for analysis.

In all experiments with expressed sap the escape of carbon-dioxide will be a source of error, especially where a bicarbonate system is present, as the bicarbonate-carbonic acid ratio in the uninjured cell may differ from that which will result when the carbon-dioxide content forms an equilibrium with the air.

A phosphate solution containing a 0·1 gramme molecule of KH_2PO_4 and a 0·1 gramme molecule of K_2HPO_4 per litre was prepared. This solution was of 0·2 M concentration and the reaction was of pH 6·6. The addition of 0·5 c. c. N HCl shifted the reaction to pH 5·8; 0·6 c. c. N HCl brought the pH from 6·6 to 5·6. The phosphate solution was now approximately 0·19 molar. This 0·19 molar phosphate solution was brought into contact with CO_2 gas as in previous experiments with Sunflower sap (p. 216).

The following shifts in reaction were obtained. —

Table XXIII

Reactions of a 0·19 M. Phosphate solution in contact with varying percentages of CO_2.

°/₀ CO_2	0	20	30	40	50	90	96
pH	5·6	5·6	5·4	5·4	5·4	5·4	5·4—5·2

°/₀ CO_2	0	30	40	50	60	70	80	90	96
pH	5·8	5·8	5·8	5·8—5·6	5·8—5·6	5·6	5·6	5·6—5·4	5·4

Previous to the addition of any hydrochloric acid 96 per cent. CO_2 shifted the reaction of the ·2 M phosphate solution from pH 6·6 to pH 6·4—6·2.

The expressed sap of Bean stem was then tested for CO_2 effects in a similar manner (Table XXIV).

Table XXIV

Reactions of the Expressed Sap of Bean Stem with increasing percentages of CO_2.

°/₀ CO_2	0	5	10	20	30	40	50	60	70	80	90
pH	5·8	5·6—5·4	5·4	5·2	5·2—5·0	5·0—4·8	4·8	4·8	4·8	4·8	4·8

The buffer value of a sample of this sap was 0·017 M between pH 5·8 and pH 6·4. The H_3PO_4 content was 0·0103 M. Between pH 6·2 and pH 6·4 there was an increase of buffer value equal to that of a 0·002 molar phosphate solution. A phosphate solution of one-tenth the previous concentration was, therefore, prepared and tested with carbon dioxide as before (Table XXV).

Table XXV

Reactions of a 0·019 molar phosphate solution with increasing percentages of CO_2.

pH	0	5	9	10	20	30	40	50	60	70	80	90
% CO_2	5·6	5·6	5·4—5·2	5·2	5·0—4·8	4·8	4·8—4·6	4·6	4·6	4·6	4·6	4·6– 4·4

The CO_2 effect was slightly greater on this weak phosphate solution than on the sap of Bean. This is what we should expect because of the greater complexity of the buffer conditions within the sap.

Even with the more concentrated phosphate solution carbon-dioxide in percentages above 30 produces shifts in reaction, while with phosphate content approximately equal to or slightly higher than actually found in Bean percentages of CO_2 from 5 per cent. upwards produce such shifts as are tabulated above.

SUMMARY

The inorganic phosphate content of the expressed sap of Broad Bean, stem and root, was determined by EMBDEN's method. The buffer value of the expressed sap was determined by titration with standard alkali and the results so obtained were calculated in terms of molar phosphoric acid.

Stem and root sap exhibited similar phenomena: — (1) variability of the amounts of inorganic phosphates present; (2) variations in the amounts of buffer action; (3) at all the reaction points determined the sap of the stem or of the root, exhibited a higher buffer capacity than that due to the contained inorganic phosphate; and (4) in all cases the buffer value of the sap increased as titration proceeded towards an arbitrary end point, which was pH 6·8 in most cases; (5) the differences between H_3PO_4 content and buffer values, at the various reaction points determined, apparently bore no definite relation to the actual amounts of inorganic phosphates in solution in the cell-sap.

Preliminary qualitative tests for organic acids indicated the presence of oxalates and malates in the sap.

The oxalic acid was precipitated as calcium oxalate and estimated quantitatively by titration with standard permanganate solution. The presence of 1·9 grammes oxalic acid per litre of sap was determined.

The malic acid was determined gravimetrically, the acid being precipitated as calcium malate. Varying quantities, from 1·733 to 2·125 grammes of calcium malate per litre of sap, were obtained. A malate solution containing 1·553 grammes of malic acid per litre had a buffer capacity, between pH 5·8 and pH 6·0; corresponding to that of a 0·00256 M phosphoric acid solution.

On the acid side (pH 5·8 to pH 5·2) of its normal reaction the sap of Bean stem exhibited a higher buffer value than on its alkaline side, (pH 5·8 to pH 6·2) to the extent of a 0·0015 M phosphoric acid solution.

Oxalates act as buffers within the reaction range pH 3·0 to pH 5·3. Malates buffer between pH 3·7 and pH 6·0. Inorganic phosphates are effective as buffers from pH 5·2 to pH 8·0.

Titration with alkali indicated the presence of some system, other than the phosphate, capable of stabilising the reaction of the sap on the alkaline side of pH 6·0.

The possible effects of proteins and their derivatives, in this region, are considered.

The addition of a concentrated solution of tartaric acid to the sap caused evolution of bubbles of CO_2, indicating the presence of carbonates in the sap.

The amounts of carbon-dioxide obtained, at room temperature and at atmospheric pressure, varied from 3 c. c. to 7·5 c. c. per 100 c. c. sap.

A bicarbonate-carbonic acid system buffers from approximately pH 5·8, to a reaction point on the alkaline side of pH 7·0.

Beyond pH 5·8 in the more acid direction any carbonates present will exist as free acid and as such may produce comparatively large shifts in reaction, and may also in the living cell take part in the formation of organic substances which may alter the buffer value of the sap.

In contact with CO_2 of concentrations ranging from 5 per cent to 50 per cent, the reaction of the expressed sap of Bean was shifted by increasing amounts; 50 per cent CO_2 brought the reaction from pH 5·8 to pH 4·8. Increasing percentages of acid gas gave no further shift in pH value.

The effect of CO_2 on a 0·019 molar phosphate solution was determined. The reaction was shifted slightly more than in the case of the expressed sap owing probably to the greater complexity of the conditions within the sap; 50 per cent CO_2 moved the reaction from pH 5·6 to pH 4·6, while 90 per cent produced a very slightly greater shift to pH 4·6—4·4. With stronger phosphate solution, 90 per cent CO_2 altered the pH figure of a 0·19 M phosphate solution from pH 5·6 to pH 5·4 so that even with this more concentrated phosphate solution CO_2 produced some effect.

It is necessary to remember that the state of affairs, including buffer conditions, the physical and chemical states of numerous substances &c., may be even more complex within the living cell than in the expressed sap. Unfortunately, investigation of such phenomena as buffer action in the living cell is not possible as yet.

This is the work as presented by Miss MARTIN without the use of the Buffer Index. The Broad Bean, as may be seen from the above, presents a much more complex buffer problem than did the sunflower. *Vicia faba* shows probably a closer approach to normal buffer conditions than the sunflower, but the low buffer values are still noteworthy in comparison with animal fluids, and carbon dioxide is still an active determining factor in the natural reaction range of pH 6—6·2. The malate and oxalate buffering is probably responsible for the absence of acidification in the under-ground parts of the stem in the bean.

The buffer systems identified are phosphates, carbonic-acid-bicarbonate, malic-acid-malate, and oxalic-acid-oxalate.

3. BUFFER INDEXES IN VICIA FABA

In the presence of only one buffer system, as in the sunflower, the use of buffer values in terms of the change of the ratio of the two components of the single buffer system is quite legitimate and presents the phenomena quite clearly. In the presence of more than one buffer system it is extremely difficult to present the phenomena clearly other than as a series of Buffer Index curves. Miss MARTIN was able to indicate other systems, in addition to the phosphate system, which buffer in the stem juice of *Vicia faba*; but the interactions are too complex to be described adequately without a series of actual curves.

Nevertheless, she determined *quantitatively* the extent to which all these systems are present. If the data in Table X be taken for a buffer index curve of the juice from the stem, we get three points on a curve. A fourth point can be obtained by calculation from the acid-titration data given on p. 250. The Buffer Index curve for the stem juice is given in figure 19. The phosphate content of the same juice was ·0134 M. The average values for the other systems can be calculated from the data given (pp. 260—261) and are — Malate ·011 M; Oxalate ·021 M; Bicarbonate ·005 M.

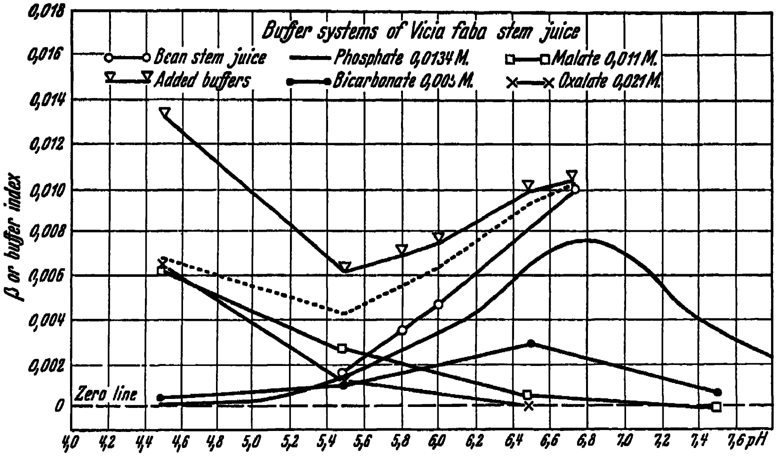

Fig. 19

The Buffer Index curves for all four systems when present in these concentrations are given separately in figure 19. Phosphates are given for 0·2 changes of pH, the others are given for 1·0 changes of pH so that the last three curves are somewhat flattened as compared with the phosphate curve. This error of grouping prevents an accurate analysis of the curves, but it will be readily seen that, in the range of the curve for the juice (pH 5·5 to pH 6·8), the malate and oxalate exert a very small degree of buffering. On the other hand, both phosphate and bicarbonate rise as does the value for the juice and both pass nearly to zero at the left ends of the curves. Further, if the buffer indexes of the systems known to be present are added together we get a curve which is similar to that for the juice, not only qualitatively in form and position on the pH scale but within limits quantitatively. The figures are of the same order of magnitude but those of the "added buffers" are higher along the whole length. The difference, which is rather large around pH 5·5, diminishes to about 40% around pH 6·0 and to about 2% around pH 6·8. Considering the errors of grouping and the experimental errors with minute precipitates and with small quantities of "base added" the agreement is reasonably satisfactory. The phosphate values and the phosphate curve can be taken as reasonably accurate. The phosphate system is, therefore, clearly the main buffer system in the stem juice of the broad bean between pH 5·5 and pH 6·8. The divergence of the curve for the juice, from the phosphate curve, cannot be accounted for by the carbonic-acid-bicarbonate system being present in the sample of Table X in smaller than the average concentration obtained for other samples of juice. The malate and oxalate systems would come into operation to an increasing extent below pH 6·0 and the concentration of these acids in the different samples was found to vary; a reduction of the malate and oxalate concentrations to one-half of the values given would result in a very close agreement (see dotted line in figure 19). It should now be obvious why such stress was laid in Chapter IX upon the recording of the quantitative data necessary for the calculation of Buffer Index curves. By the use of these curves the complexity is made clear and comprehension of the phenomena follows naturally.

The "iso-electric" form of this series of curves is discussed in Chapter XIX, and a better scheme for quantitative determination of organic acids is given in Appendix II.

CHAPTER XIV

THE POTATO — (SOLANUM TUBEROSUM)

TISSUE REACTIONS AND BUFFERS

The tuber of the potato plant has entered as material into many investigations.

WAGNER (1916) records pH 5·9 for the extracted tuber juice, with a rise to pH 6·0 followed by a decrease to pH 5·7—5·3 on infection with *Bacillus phytophthorus.* WEISS and HARVEY (1921) give H-electrode data (residual pH values as usual) for quite a number of varieties, using expressed juice of tubers. These values range from pH 6·67 to pH 6·17 for normal plants and pH 6·11 to pH 5·90 for plants infected with *Bacillus tumefaciens.*

STOKLASA (1924) gives pH 7·0 for the pressed juice of potato *roots.* PEARSALL and EWING (1924) give pH 5·4—5·6 for expressed *tuber* juice, passing to pH 6·2 after filtration. YOUDEN and DENNY (1926) give pH 6·10 for tuber juice and pH 6·41 for a water extract of the tissue.

HERKLOTS (1924) records a value between pH 3 and pH 4 for the suberin block of wounded tubers. Working on wound reactions, HERKLOTS (*ibid.*) found that buffered solutions or jellies of pH 4·2—7·0 were toxic to the general tissue, while pH values above pH 7·0 gave no killing of the tissue. Alkalinity (from pH 7·5) was found to promote suberisation and retard meristem activity; while acidity (down to pH 4·6) was found to promote meristem activity and to retard suberisation. Acetate buffers were exceptionally toxic.

SAMUEL (1927), without giving actual figures, states that a pH map of the potato tuber could readily be made out, the vascular circle and the tip being most acid. He used a microspear with solid quinhydrone and the wound-effects of this method would appear to be considerable, see below under "Wound Carbon Dioxide".

Considering the methods used, a hydrion concentration in the range pH 5·6—6·2 seems indicated for the expressed tuber juice of potato, the variations being due to variation in manipulation rather than to natural variations of the tissue in its living condition.

1. TISSUE REACTIONS

These have been investigated by the writer (*solo*). All determinations have been made, using the improved R. I. M. technique, on the resting tuber (var. British Queen) and on tubers sprouted in pots during the winter in a heated greenhouse. Aqueous indicator solutions (except BAN) maintained in an accurately balanced condition were used.

a) MACROSCOPIC OBSERVATIONS

When a clean tuber was cut across, the following indicator colours were obtained on the freshly cut surface — BTB yellow; BCP yellow becoming pale purple externally only; DER pink becoming yellow; MR pinkish orange becoming yellow. This flushing pink seemed to indicate the passing away of carbon dioxide.

Wound Carbon Dioxide. — The production of an evanescent excess of carbon dioxide over the normal CO_2 content, when the tuber tissue is injured, can be demonstrated easily by (1) flooding the freshly cut surface with DER or MR, (2) allowing the pink flush to pass away, and (3) drawing the back of a penknife hard across the now yellow surface. A livid red streak appears along each cut, indicating a pH $<$5·2 with MR. That this is due to a volatile acid is shown by the gradual disappearance of the red colour and a return to yellow. Wound carbon dioxide is thus indicated, as increasing the $[H^{\cdot}]$ of the fluid of the crushed tissue to pH $<$5·2.

Cutting and Injury. — An elegant distinction may be made between cutting open one layer of cells and injury by crushing, using BCP and DER. The cut surface flooded with DER becomes gradually yellow (pH $>$5·9), with BCP it becomes clearly pale purple on the surface (pH $>$6·2), but yellow immediately below the surface (pH $<$5·9). If now a razor edge be drawn very gently across the DER yellow there is no change, no reddening; but similar treatment of the BCP pale purple surface gives a fine line of yellow, returning to pale purple within less than a minute. A deeper cut with the razor gives again a yellowing with BCP, but also a reddening with DER.

The cutting open of one layer of cells allows sap (with CO_2) at pH 5·9 ca. to escape, yellowing the BCP but not reddening the DER. Injury, on the other hand, by a deeper cut or a crushing of the cells, results in the production of wound carbon dioxide in such concentration that the reaction is swung below pH 5·2. The concentration of carbon dioxide required for this change is comparatively large, about 20—40 %, see p. 289, but this is not much above the maximum intercellular carbon dioxide content found by Magness (p. 331).

The Effect of Sectioning. — These observations would suggest that the effects of taking a section of living plant material are — (1) an evanescent wound reaction, giving wound carbon dioxide in quantity; (2) the passing away of the consequential increase in $[H^{\cdot}]$ with a return of the living tissue apparently to normal; and (3) that, if a sharp razor be used with care to avoid crushing of the cells as distinct from sectioning, this wound reaction can be taken as passing away within at most 2—5 minutes after the sectioning. Consider, however, the effect produced by crushing the tissues to express the juice. The subsequent reaction might well vary from pH $<$5·2 to pH 6·2 according to the degree to which the wound carbon dioxide was removed during the manipulation of the juice. This crushing effect explains the variation from pH 5·4 initially to pH 6·2 after filtration which was found by PEARSALL and EWING.

The effect of sectioning can be studied macroscopically, using transverse sections of the middle region of young shoots. If the sections be taken and placed immediately in aqueous diethyl red, the whole section becomes rosy pink. This pink in a minute or two passes away leaving the sections mainly yellow with a pink epidermal margin. The sections may be left for at least four hours in a watch glass of DER solution and still remain yellow. They also remain a general yellow if placed upon a glass slide and kept covered with DER solution; but if a cover-slip be placed over the section a general pink colour appears all through the section within five or ten minutes, and the indicator in the *immediate* neighbourhood of the section may also be affected. The same acidification on mounting a section under a cover-glass may be noted with BCP.

Since this acidification is reversed, the pink DER or yellow BCP reverting to yellow DER and pale purple BCP, when the cover-glass is removed, it seems probable that we are here dealing with the normal carbon dioxide production of respiring non-green cells, rather than with an injury effect. If the cover-slip be pressed down on the section so that the latter is squashed there is, of course, injury accompanied by acidification which passes away but this acidification cannot be repeated as it can with a non-injured section.

These observations indicate that one effect of sectioning may be to allow the respiratory carbon dioxide to diffuse away more readily than it does in the whole tissue, in which case many of the R. I. M. records of A and a ranges may represent e in the natural living tissue. In any case these observations demonstrate that respiratory carbon dioxide cannot be neglected in a critical consideration of the internal pH of living cells, especially those which have a sap reaction in the range pH 5·6—6·2.

b) MICROSCOPIC OBSERVATIONS

Subject always to the above-mentioned carbon dioxide error of sectioning, the following records may be taken as more critically near to the natural hydrion concentration than most of the records yet published, since all the precautions, which have been shown to be advisable, were put into practice.

TUBER

Reserve Tissue. — A certain amount of self-colour, pink in the cells, was found in the outer cortex of the tuber. The parenchymatous tissue gave reactions indicating pH 6·2, but there seems little doubt that the real internal pH with the normal intercellular carbon dioxide content is at least as low as pH 5·9. Left over-night in aqueous indicators this tissue shows pH 6·2 in the contents and range a in the walls very distinctly. The purple and yellow differentiation of contents and wall with BCP is quite striking. The lignified xylem walls throughout are in range e. The phellogen is pH 5·9; the normal cork cell-walls are range e and the walls of suberised wound tissues are k. The tissues of the eye-bud in the resting tuber appear to be pH 5·9 also, with the exception of the acid apiculus, see below.

Eye. — The very young eyes of early stages of sprouting show a small apiculus and a thicker portion of stem with numerous adventitious rootlets arising from the pericycle. The apiculus in longitudinal section shows a core of pH 5·9, surrounded and covered at its apex by a sheath of acid tissues (pH 5·2—4·8). Transverse sections near the apex show that all tissues except the vascular ring are in range e, including the pith. The xylem vessels have the usual acid walls.

The thicker part of the eye stem shows the epidermis, subepidermis and parts of the outer cortex acid, pH 5·2—4·8; the xylem walls are acid as usual, and so are the lignified walls of the more or less isolated fibres which occur in both the external and internal phloem regions.

The rootlets show a hollow core of pH 5·9, with all the outer tissues acid, e: The central xylem strand is also in range e.

Older eyes or very young sprouts showed similar features, but with the eye stem 1—1·5 cms. long there is some variation from top to bottom thus.

	Epid.	Sub-ep.	Cortex		Vasc. ring	Pith
			Outer	Inner		
Apiculus						
tip . . .	e	e	e	e	a	e
mid . . .	e	e	d	d	a	d
bottom .	e	e	e	e	a	d
junction .	e	e	e	e	a	e
Thick part						
top . . .	e	e	e—a	a	a	e
mid . . .	e	e	e—a	a	a	a
bottom .	e	e	e	a	a	a

The rootlets in these older eyes show both dermatogen and cortex pH 5·2—4·8 with the vascular core at pH 5·9, except for the acid xylem walls.

SAP, CYTOPLASM AND WALL. PLASMOLYSIS AND MICRO-DISSECTION

The walls where they can be stained indicate range a for parenchyma; lignified and suberised walls are e normally and k for wound tissue.

The cells with acid contents were further investigated. The red coloured contents (with DER and MR) could be plasmolysed and deplasmolysed repeatedly with 0·5 molar sucrose solution. The wall could be seen yellow, as distinct from the acid contents. The acidity would, therefore, appear not to be due to carbon dioxide.

Using a pair of CHAMBERS' Micromanipulators, it was found that one cell at a time could be punctured, and on each occasion the red-dyed sap could be seen to escape as a small cloud of red colour into the yellow indicator solution where it quickly changed colour and disappeared from view. At the same time the cytoplasm could be seen left in the punctured cell and it was always yellow. This differs from SCHAEDE's findings with injured onion cells (see Chap. XVI), but it was confirmed repeatedly. In this case, therefore, the wall and contents have been separated by plasmolysis, and the contents have been separated as sap and cytoplasm by microdissection. We can say with some degree of certainty that the wall is in range a, the cytoplasm is of pH 5·9, and the sap is of pH 5·2—4·8.

STEM

Throughout the aerial stem of young shoots 15 to 20 cms. long, all the tissues are yellow (acid) with BTB (pH <6·2), and show the alkaline colours for BAN, BCG and BPB (pH >4·8). Differentiation is obtained with BCP, but all the pale purple records are suspected of carbon dioxide error. Differentiation is also obtained with DER and MR; so that it is with these last two indicators that we get the apparent differences giving ranges a (pH 5·9), b (pH 5·9—5·6), c (pH 5·6) and e (pH 5·2—4·8).

Base of Stem. — This part shows stem buds and rootlets, and the stem itself indicates thus: —

Epid.	Sub.-ep.	Outer Cortex	InnerCort.	Endodermis	O.Phl.	Xyl.	I.Phl.	Pith
e	e	e in parts a ,, ,,	a	a	a	e	a	a e

with e for the bast fibre walls in both outer and inner phloem. The rootlets are in range a, except the dermatogen (piliferous layer), outer cortex and central xylem strand which are in the range e. The outer cortex was very distinctly acid around the base and near the apex of the rootlets. The stem buds had all the tissues a, except the epidermis and sub-epidermis which were e. Clothing hairs were a.

Middle of Stem. — This part of the young stem was distinctly less acid than the basal part. The epidermis was c, instead of e, the sub-epidermis and outer cortex were a instead of e, since the sections were all yellow with MR except for lignified walls in xylem and bast fibres. The clothing hairs were in range a, but the heads of the glandular hairs varied, being a when young but c when older.

It was with this part of the young stem that the critical observations on respiratory carbon dioxide were made. Microscopic observations showed that the cambium in particular turned pink rapidly with DER, but all the other living cells changed colour also, when a cover-slip was used. The real natural pH of these tissues would, therefore, appear to be c (pH 5·6) or e (pH 5·2—4·8) rather than the a (pH 5·9) obtained by observing sections without a cover-slip. This reasoning would not apply to the hairs in the a range, nor to the epidermis when it was at c or possibly also at e.

Carbon dioxide content is clearly a master factor in determining the natural pH of all the *internal* living tissues of the young potato stem in this middle region. Above and below the middle part organic acids may account for the more acid sap in some tissues such as the epidermis, hypodermis and outer cortex.

Top of Stem. — The upper part of the aerial stem was very similar to the middle part, but the sub-epidermal layer and one or two layer sof the outer cortex were c instead of a. The epidermis and other tissues were the same as for the middle.

LEAF

The petiole showed the contents of epidermis and sub-epidermis in the e range, the usual acid walls for the xylem and the rest of the tissues at pH 5·9. The mesophyll, being deep green, showed no clear indication, while the midrib (rachis) was similar to the petiole. Self-colour was present in some non-chlorophyllous parts but not in all.

SUBTERRANEAN PARTS

Etiolated base of Aerial Stem. — This part of the stem showed a very distinct differentiation with DER. Again all tissues were in the range pH 5·9—4·8. The walls of the xylem vessels and of the numerous bast fibres of both outer and inner phloem gave bright reds with DER and MR (range e). The pith and practically all the cortex were in the range pH 5·9—5·6; all yellow with MR and BCP, varying from cell to cell with DER, but showing mostly yellow (pH 5·9 *ca.*). The epidermis, sub-epidermis and some of the outermost cells of the cortex were acid (pH 5·2—4·8), as in the base of the stem above ground.

The most striking phenomenon, however, was the very distinct pink with DER throughout the whole of the vascular ring. Outer and inner phloem, xylem parenchyma and cambium all gave clear pink with sections which had been washed in neutral water and immersed in DER solution. With MR there was again a decided pink throughout the vascular ring, but this pink faded fairly quickly and gave way to a yellow. The vascular ring in fresh sections is, therefore, of pH 5·2—4·8, quite apart from the wall reactions of vessels and bast fibres which take the stain more deeply and persistently. The passing away of the pink with MR would indicate either an escape of carbon dioxide or the presence of some other readily diffusing acid in a concentration just sufficient to give pH $<5{\cdot}2$. The pink with DER, although it persisted longer than that with MR, did pass away within about two hours, the red colour of the epidermis contents and lignified walls persisting for at least twelve hours.

This evidence would indicate that the epidermal acidity is due to organic acid which does not diffuse very readily, while the acidity of the internal tissues is mainly due to carbon dioxide.

RHIZOME

Very young tuber-producing stem branches showed range e in contents of epidermis, sub-epidermis and the outermost cortical cells. Most of the cortex and all the pith was of pH 5·9. The living cells of the vascular ring, including the cambium, were of pH 5·6, the xylem walls and fibre walls were e.

The older tuber-producing stem branches closely resembled the etiolated base of the aerial stem, but the vascular ring was in range **d** (pH 5·6—4·8) rather than e, since the pink with MR was not very definite and at best it was distinctly fugitive. The actual reaction is possibly between pH 5·2 and pH 5·6. The cortex and pith again varied from cell to cell but showed almost as many cells at pH 5·6 as at pH 5·9.

ROOT

The central xylem strand showed e, with xylem parenchyma and phloem at pH 5·6 and the layer between (cambium in part) at pH 5·9. The cortex was at pH 5·9 and the piliferous layer at pH 5·6.

c) SUMMARY

These observations indicate that in *Solanum tuberosum* —

1. The outer layer, epidermis or piliferous layer, together with one or more of the adjacent layers, has an acid sap, sometimes pH 5·2—4·8, but rising in the upper parts of the aerial stem and in the older rootlets to pH 5·6. This acid (e) layer is the whole depth of the cortex in the young tips of roots and in the apiculus of the young eye shoot.
2. The cytoplasm is, when seen separated, at pH 5·9.
3. The reaction of the lignified and suberised walls is in range e, or for wound suberin range k. The cellulose walls may be in range a.
4. The actual pH of the other tissues lies within the range pH 5·9—4·8, and appears to be dominated almost entirely within that range by the carbon dioxide content of the cells and intercellular spaces. The cortex and pith are frequently of pH 5·9 in free sections, but this is shown to be subject to a carbon dioxide error, and the real value in the living cortex and pith throughout (with the exception of the very young root and the apiculus of the eye) is suggested as pH 5·6. The reaction of the vascular ring in aerial parts of the plant would appear on the same evidence to be also pH 5·6, but in the etiolated subterranean parts of the stem the natural reaction would appear to be slightly below pH 5·2.
5. Acidity in the tissues of the potato seems to be caused by (a) wall acids, (b) organic acids in the sap of outer tissues, and (c) carbon dioxide in the internal tissues.

2. BUFFERS

The buffer capacity of tuber and leaf, and the buffer systems present in the potato tuber were reported upon by C. T. INGOLD (1929) as follows.

The present work was undertaken to determine the extent of the buffer action in the potato (*Solanum tuberosum*) and the nature of the buffering systems.

BUFFER INDEX

In the previous work on buffering in this series the degree of buffering of the sap is measured by the concentration of inorganic phosphate necessary to give the same degree of buffering. While this method was particularly useful in dealing with the case of Sunflower, it does not give a generally useful measure of buffer capacity since the titration curve of phosphoric acid with alkali is not a straight line but a series of more or less flat parts connected by much steeper portions.

As a measure of the buffer capacity the "Buffer Index" of VAN SLYKE was taken. This is defined [see KOLTHOFF (1922), p. 24] as

$$\beta = \frac{dB}{dpH}$$

where β is the Buffer Index and B is the acid or alkali added. In this paper the equation is used in the form

$$\beta = \frac{\text{Number of gram equivalents of acid or alkali added to one litre of solution to produce a certain shift in pH}}{\text{the shift in pH produced}}.$$

In stating β the range must also be given, since β for a particular solution varies over the pH scale. The Buffer Index is really a standard way of expressing the degree of slope in the titration curve.

To obtain the buffer-index curve it is first necessary to construct a titration curve. These curves in this work were obtained by titrating the sap or other solution with acid (HCl) and alkali (NaOH), the pH determinations being made by means of a B. D. H. Capillator. Using the indicators PR., BTB., BCP., MR., BCG. and BPB. titrations could be carried out between the points pH 3·2 and pH 7·6. The colour of the sap did not interfere with the capillator determinations. The experimental error in determining the pH was roughly ±0·1.

Once the titration curve is obtained the buffer indices can be calculated between pH 4 and 5, pH 5 and 6, and pH 6 and 7. In a particular case in which portions of sap, each of 5 ccs, were titrated with 0·05 N HCl and 0·05 N NaOH the titration curve showed that the amount of acid or alkali required to shift the reaction from pH 4 to pH 5 was 2·9 ccs, from pH 5 to pH 6 1·4 ccs and from pH 6 to pH 7 0·95 ccs.

To work out the buffer index between pH 4 and 5 there are the data:

2·9 ccs of 0·05 N acid required to shift the reaction of 5 ccs from pH 5 to pH 4.

Then the Buffer Index,

$$\frac{\dfrac{0{\cdot}05 \times 2{\cdot}9}{1000} \times \dfrac{1000}{5}}{1} = \frac{\begin{array}{c}\text{Number of gram equivalents of acid}\\ \text{required to be added to 1000 ccs sap}\\ \text{to shift reaction from pH 4 to 5}\end{array}}{\text{the shift in pH}}$$

$$= 0{\cdot}029.$$

Similarly for the ranges pH 5 to 6 and pH 6 to 7 buffer indices are respectively 0·014 and 0·0095.

Hurd-Karrer (1928) in a recent paper uses the reciprocal of the buffer index $\frac{dpH}{dB}$, but it appears to the writer, his colleagues and most other workers on buffer capacity that such results are much more clearly presented when put in the form of a Buffer Index Curve with pH ranges as abscissae and β values as ordinates.

EXTRACTION OF THE SAP

Peeled tubers of the variety „British Queen“ were used throughout the work except where otherwise stated.

The sap used for the experiments was obtained by squeezing the tissue in a specially devised press. The crude sap was cleared by filtration through a layer of asbestos on a Buchner funnel. The asbestos pad was washed with distilled water before passing the sap through. The liquid thus obtained was free from visible suspended matter but was of a bright brown colour due to an oxidase reaction in the sap.

It might be mentioned in passing that the buffer indices obtained may conceivably vary with the manner in which the sap is extracted. Thus Dixon and Atkins (1913) found that the solute content of the first pressings from plant tissues was considerably lower than in later pressings. Again Hoagland and Davis (1923) compared the sap obtained from *Nitella* (a) by breaking the cells and collecting the sap which oozed out and (b) by squeezing out the sap in a screw press, and filtering the resulting fluid. They found that the electrical conductivity of the sap collected by breaking the individual cells and pouring out the contents was 50 % greater than the conductivity of the expressed sap.

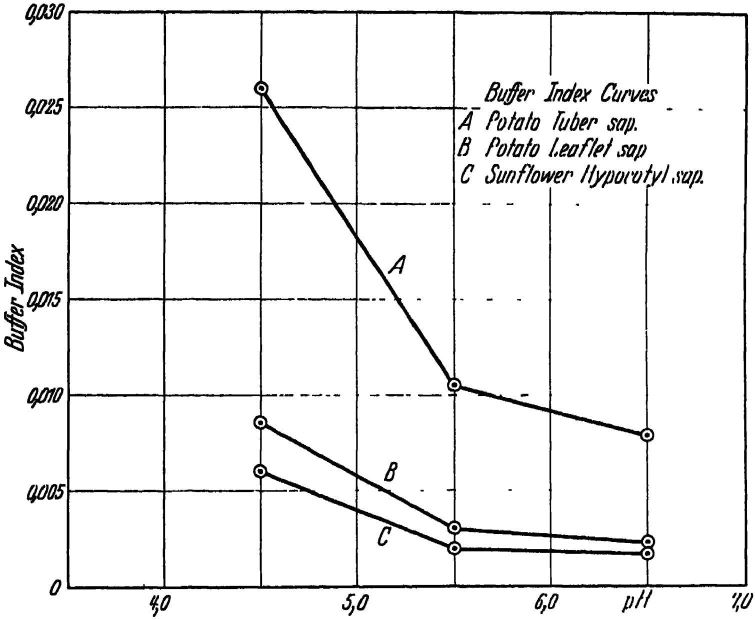

Fig. 20

THE BUFFER INDEX CURVE

Figure 20 gives the buffer-index curve of the sap of the tubers. This is an average of determinations which are given in detail in the following table.

Table I

Buffer indices of samples of "seed" tubers

Sample	pH range		
	pH 4 to 5	pH 5 to 6	pH 6 to 7
A	0·034	0·009	0·008
B	0·0212	0·010	0·0075
C	0·025	0·0084	0·0070
D	0·029	0·014	0·009
E	0·021	0·011	0·008
Average	0·026	0·0105	0·0079

It may be seen from this table that there is considerable variation in the buffer indices of the sap from sample to sample but that in each case the general form of the curve is maintained, *i. e.* the buffer index rises with decreasing pH. In figure 20 the buffer index curve of the sap of the leaflets of the mature potato plant is given and also the curve for the sap of Sunflower hypocotyl.

An inspection of this figure shows clearly that the sap of the tuber is much more strongly buffered than the sap of the leaflets but that the general form of the curve is the same.

Martin (see Chap XII) has shown that in the region pH 5·6—6·8 the sap of the Sunflower is buffered solely by the inorganic phosphate present. Experiments were accordingly carried out to see whether the same was true of the potato tuber sap.

THE INORGANIC PHOSPHATE BUFFER

A casual examination of the buffer-index curve shows quite clearly that phosphates cannot be the only buffer system present. The buffer index curve of inorganic phosphate falls from pH 6—7 to pH 4—5. In contrast to this the buffer-index curve of potato sap rises steadily from pH 6—7 to pH 4—5.

The inorganic phosphate in the sap was estimated using Embden's technique as used by Martin (see Appendix I).

Table II gives the results of phosphate analysis and buffer index determinations between pH 6 and pH 7. Beside the buffer indices of the sap are placed the buffer indices of the inorganic

Table II

Number of experiment	pH of sap	Buffer index of sap between pH 6 and pH 7	Buffer index of inorganic phosphate present in sap in range pH 6 to pH 7	Molar concentration of inorganic phosphate in the sap
I	6·2	0·0062	0·0028	0·0058
II	5·8	0·0100	0·0030	0·0063
III	5·8	0·0085	0·0026	0·0055
IV	6·0	0·0055	0·0018	0·0037
V	5·7	0·0080	0·0033	0·0069
VI	5·9	0·0060	0·0019	0·0039
Average	5·9	0·0075	0·0025	0·0053
VII	5·9	0·0080	0·0024	0·0050

phosphate present calculated from the titration table of phosphate given by CLARK (1928).

For the first six determinations (I to VI) the sap in each experiment was obtained from a single tuber. For the last determination (VII) the sap was a mixed sample obtained from six tubers. The values for this sample agree closely with the averages of the values for the single tubers.

These results show that there is a considerable variation from tuber to tuber both in inorganic phosphate content and in buffer index between pH 6 and pH 7.

It is further clear that the inorganic phosphate present does not account for all the buffering between pH 6 and pH 7. In fact on the average it accounts for only 30 % of the buffer index.

This value can be compared with the other examples which have been worked out for approximately the same range of pH. In Sunflower, hypocotyl, stem and root, inorganic phosphates represent 100 % of the buffer index, in *Nitella* about 66 %, in Broad Bead stem 50—90 %, in Broad Bean root 60 – 80 %.

In the potato sap inorganic phosphates account for still less of the buffering as more acid regions are reached. Thus in the particular case given in figure 21 (boiled and filtered sap) between pH 6 and pH 7 inorganic phosphate accounts for 37 % of the buffering, between pH 5 and pH 6 for 8 % and between pH 4 and pH 5 for less than 1 %.

THE CITRATE BUFFER

The shape of the buffer-index curve of the sap suggested that some buffer should be looked for which has an increasingly stronger effect from pH 6—7 to pH 4—5. Organic acids in general show this feature. The maximum buffering occurs at a hydrogen ion concentration numerically equal to the dissociation constant of the acid. For oxalic acid it is $10^{-4\cdot46}$ (second step), for citric acid $10^{-4\cdot3}$ (second step), and $10^{-5\cdot7}$ (third step), for malic $10^{-3\cdot5}$ (first step) and $10^{-5\cdot0}$ (second step) and for succinic $10^{-4\cdot2}$ (first step) and $10^{-5\cdot2}$ (second step).

Since citric acid and citrate have been recorded as important constituents of potato juice (TIBBLES), the next stage in the investigation was a determination of the buffer action of the citrate present.

A large volume of sap was obtained, boiled, filtered and made up to the original volume with distilled water. This was divided into three portions. One part was used for buffer index determinations, another for phosphate analysis and the third for citrate analysis.

The buffer indices of the boiled sap were:

pH range . . .	4—5	5—6	6—7
Buffer index . .	0·026	0·0095	0·0062

The concentration of inorganic phosphate in the sap was 0·0049 molar and the buffer indices for this are:

pH range . . .	4—5	5—6	6—7
Buffer index . .	0·00016[1]	0·00074[1]	0·0023[2]

The method of citrate analysis used was that described by PRINGSHEIM (1910): 261 ccs of boiled sap were used for this determination.

To this sap excess of lead acetate (20 % solution) was added and the volume of the whole resulting solution doubled by adding alcohol (90 %). This was shaken and left overnight. A voluminous grey precipitate was formed. This precipitate was collected, mixed with distilled water, shaken with an equal volume of alcohol, and again filtered and washed with 50 % alcohol. The precipitate was then mixed with distilled water and decomposed

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1) Calculated from PRIDEAUX's curve [BAYLISS (1924), p. 205].

2) Calculated from CLARK [(1922), p. 107].

by passing a stream of H_2S through the heated liquid. There resulted a black precipitate of lead sulphide. This was filtered off. The filtrate was concentrated and neutralised with KOH. Then double the volume of alcohol was added and the whole was shaken and left over for a few days. The white precipitate which separated out contained some of the phosphates and sulphates. This precipitate was filtered off and the filtrate (180 ccs) was mixed with 10 ccs of 30% acetic acid. Any tartrate present should separate out at this stage as acid potassium tartrate. A very slight precipitate did form. This was filtered off and the filtrate was heated until all the alcohol was driven off. The solution was then acidified with HCl and extracted with ether. The watery solution left over from the ether extraction was neutralised with NaOH. $BaCl_2$ (10% solution) was then added giving a heavy white precipitate. The precipitate was filtered off and alcohol was added to the filtrate in the proportions 72 parts of solution to 28 parts of alcohol. A voluminous white precipitate formed on the addition of the alcohol. This is a precipitate of barium citrate which is sparingly soluble in water but insoluble in 28% alcohol. This was filtered off and washed with 28% alcohol. The precipitate was then mixed with water and a few drops of nitric acid added to aid the solution of the precipitate. Dilute H_2SO_4 was then added giving a white precipitate of barium sulphate. This was filtered off, dried and weighed. It was found to weigh 0·525 grams. Now each gram of this precipitate is equivalent to 0·548 grams of water-free citric acid.

From these data it can be calculated that the citrate is present in the sap in 0·0057 molar concentration.

The buffer indices of 0·0057 molar citrate are:

pH range . . .	4—5	5—6	6—7
Buffer index . .	0·0047	0·0037	0·0401

These values are calculated from the citrate buffer tables given by Clark [(1922), p. 113—114].

In this particular case the citrate present accounted for 22·5% of the buffering between pH 6 and pH 7, 39% of the buffer index between pH 5 and 6 and 19% between pH 4 and pH 5.

Taken together phosphate and citrate in this case account for 60% of the buffer index between pH 6 and pH 7, 47% between pH 5 and pH 6 and 19% between pH 4 and pH 5. The buffer indices of phosphate plus citrate obtained by adding together the indices for phosphate and citrate taken separately were:

pH range	4—5	5—6	6—7
Buffer index of phosphate plus citrate	0·0049	0·0044	0·0037
Buffer index of sap	0·026	0·0095	0·0062

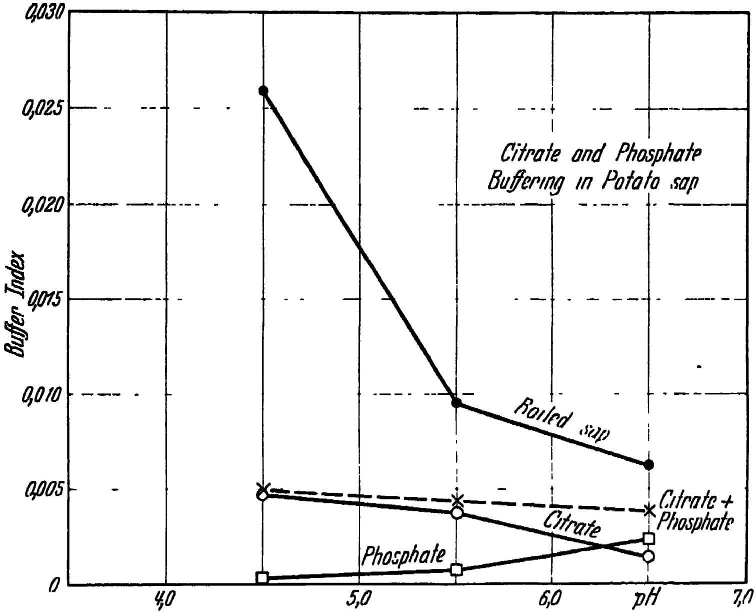

Fig. 21

It is interesting to note that the resultant buffer-index curve of phosphate plus citrate is almost a horizontal line from pH 4—5 to pH 6—7. As phosphate buffering dies out citrate buffering comes in and *vice versa*.

The relations of phosphate and citrate buffering in the sap are shown in figure 21. It is quite clear from an inspection of this figure that some important buffer other than citrate must be operating between pH 4—5 and pH 5—6. It was thought that such a buffer might be found among the ether soluble organic acids in the sap. Citric acid is insoluble in ether.

THE ETHER SOLUBLE BUFFER

In this experiment a portion of a sample of sap was taken and the buffer index curve (fresh filtered sap curve) was found.

The remainder of the sample was then boiled, filtered and made up to the original volume with distilled water. 20 ccs of this were used for buffer index determinations (boiled sap curve). The titration was as follows:

Table III

Titration of boiled sap

Volume of sample titrated	ccs 0·05 N HCl or NaOH added	Resulting pH
10 ccs	—	6·0 6·1
10 „	1·0 ccs NaOH	6·7
10 „	1·5 „ „	7·1 - 7·2
10 ccs	1·0 ccs HCl	5·6
10 „	2·0 „ „	5·2
10 „	3·0 „ „	4·7
10 „	5·0 „ „	4·3
10 „	7·0 „ „	3·9

For ether extraction 10 ccs of the boiled sap were used. This sap was first acidified with HCl, filtered (since a slight precipitate had formed) and concentrated to about 5 ccs. This was then extracted with ether making three shakings and in each case using 100 ccs or more of the ether. In carrying out the extraction vigorous shaking was continued for several hours. The ether was then evaporated and the residue dissolved in 10 ccs distilled water. The reaction of this solution was less than pH 2·8. The titration of this solution with alkali is given in the following table.

Table IV

Titration of ether extract from 10 ccs of boiled sap

ccs 0·05 N NaOH added	Resulting pH
2·0 ccs	3·0
3·0 „	3·6
4·0 „	4·2
5·0 „	4·6
6·0 „	5·1
6·5 „	5·5
7·0 „	6·1
8·0 „	> 8·0

The watery solution remaining over after extraction of the sap sample with ether was then titrated with alkali. The titration of this is given in the following table.

Table V

Titration of solution left behind from ether extraction of 10 ccs of boiled sap

Alkali added		Resulting pH
10 ccs 0·18 N NaOH		3·5
10 „ 0·18 „ „	1·0 ccs 0·05 N NaOH	3·9
10 „ 0·18 „ „	2·0 „ 0·05 „ „	4·6
10 „ 0·18 „ „	3·0 „ 0·05 „ „	5·7
10 „ 0·18 „ „	3·5 „ 0·05 „ „	6·3
10 „ 0·18 „ „	4·0 „ 0·05 „ „	7·0

By constructing the titration curves from the tables given above the buffer indices were found. The buffer indices for the boiled sap and the ether soluble and ether insoluble parts are given in table VI. As will be obvious from an inspection of table IV the sudden rise from pH 6·1 to >8·0 in the titration renders the buffer index of the ether soluble part between pH 6 and pH 7 somewhat uncertain.

Table VI

pH range	Buffer index		
	Boiled sap	Ether soluble substances	Ether insoluble substances
4—5	0·0220	0·0105	0·0065
5—6	0·0100	0·0055	0·0042
6—7	0·0060	? 0·0030	0·0038

The results of this experiment are shown graphically in figure 22.

It is clear that the ether soluble substances in the acidified sap represent important buffers, in fact the principal buffer substances in the acid region pH 4—5. The more or less horizontal line of the buffer index curve of the ether insoluble part probably corresponds to the citrate-phosphate curve to which reference has already been made (see figure 21).

The other extract was decidedly acid, being below pH 2·8. This suggests the presence of organic acids. Qualitative tests did, in fact, reveal the presence of oxalic acid in the extract[1]).

BUFFERING DUE TO ASPARAGIN

Asparagin is the principal amino compound in the potato tuber. It is present to the extent of 0·3 % in the tuber [MILROY (1921), p. 95].

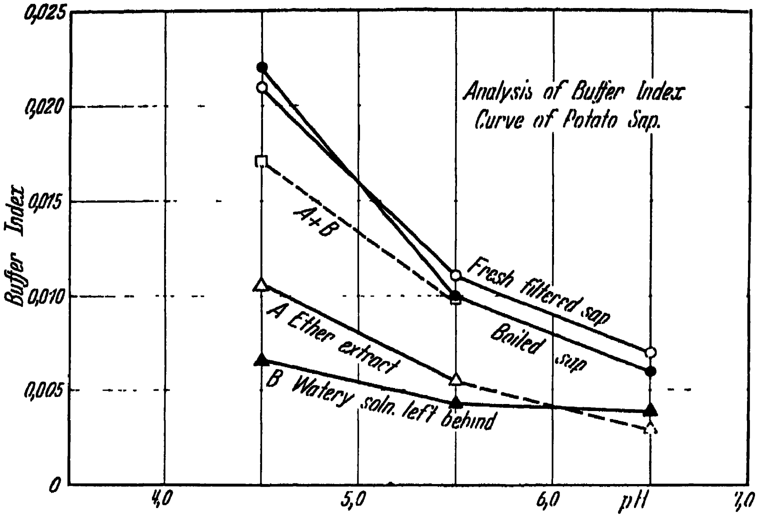

Fig. 22

A 1 % solution of asparagin was made up and the buffer indices determined. They are given in the following table.

Table VII

pH range.	4 - 5	5 6	6 7
Buffer index of 1 % asparagin	0·0008	0·0003	0·0013
Buffer index of 0·3 % asparagin	0·00024	0·00009	0·00029
Buffer index of sap	0·0026	0·0105	0·0079

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1) This and other possibilities are now being investigated and will be reported upon later.

It is clear from this table that between pH 4—5 and pH 5—6 0·3 % asparagin present in the sap would account for less than 1 % of the buffer index. In the region pH 6—7 such a concentration of asparagin would account for 5 % of the buffering. The state of affairs is shown graphically in figure 23.

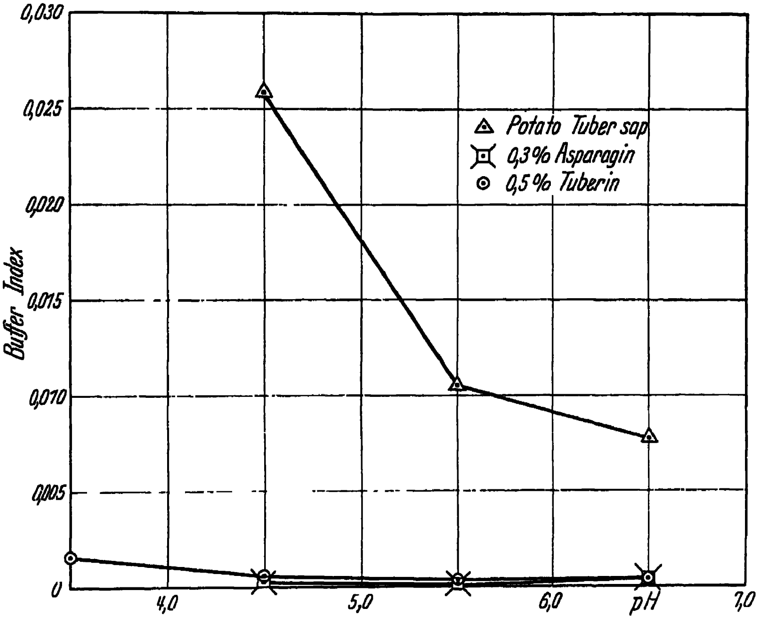

Fig. 23

BUFFERING DUE TO TUBERIN

Cohn, Gross and Johnson (1919) investigated the tuberin of potato juice. From their experiments they concluded that the tuberin was a very important buffer in the juice.

Commenting on the titration curve of the potato sap they say:

"The strong buffer action, indicated by the steepness of the titration curve throughout the range investigated cannot be attributed merely to the presence of phosphoric acid nor to the other organic or inorganic weak acids which chemical analysis reveal. The increase in the steepness of the titration curve in the range acid to pH 4·5 on the one hand and alkaline to pH 8·5 on the other, is largely due to the dissociation of the protein compounds that exist in the potato and their recombination with strong acids and bases with the retention of the hydrogen and hydroxyl ions".

On boiling potato sap a precipitate consisting probably of denatured protein separates out. The weight of this precipitate was determined and the results are given in table VIII.

Table VIII

Volume of sap sample	Dry weight of the precipitate obtained on boiling the sap	Expressed as percentage
ccs	gms	%
83	0·348	0·42
124	0·412	0·33
101	0·505	0·50
53	0·160	0·30

The heat coagulum when filtered off left a clear solution from which no precipitate separated out on addition of tri-chlor-acetic acid. In another experiment the precipitate obtained by adding tri-chlor-acetic acid to 12·5 ccs of fresh filtered sap weighed on drying, 0·020 gms corresponding to a protein content of 0·16 %.

COHN [see OSBORNE (1924)] gives a titration curve for tuberin from which it is possible to calculate the buffer indices. These are given in the following table side by side with the buffer indices of potato sap.

That the substances precipitated on boiling the sap have little or no influence on the buffer action is also shown by a comparison of fresh sap with boiled and filtered sap.

Table IX

pH range	Buffer index of 1 % tuberin solution Calculated from COHN's curve	Buffer index of potato sap
3—4	0·0033	·
4—5	0·0011	0·026
5—6	0·0006	0·0105
6—7	0·0008	0·0079
7—8	0·0011	—

In these experiments each sap sample was divided into two parts. One was titrated with acid and alkali and the other was boiled, filtered, made up to original volume with distilled water and then titrated. In this way for each sample the buffer indices of the boiled and the fresh sap were obtained. The results for four samples are given in table X.

Table X

Buffer indices of sap of young tubers

pH range	Sap sample	Unboiled	Boiled	Difference
4—5	A	0·034	0·032	—0·002
	B	0·0265	0·0265	±0
	C	0·029	0·026	—0·003
	D	0·030	0·036	+0·006
5—6	A	0·016	0·0145	—0·0015
	B	0·0145	0·013	—0·0015
	C	0·017	0·019	+0·002
	D	0·021	0·014	—0·007
6—7	A	0·0107	0·010	—0·0007
	B	0·0090	0·0075	—0·0015
	C	0·008	0·009	+0·001
	D	0·010	0·01	±0

It is clear that there is no constant difference between the two sets of readings in any pH range considered. The conclusion is that tuberin, or at any rate that part coagulated by heat, has very little effect in buffering the sap.

Hurd-Karrer (1927) working with Wheat sap came to the same conclusion namely that the protein present had a negligible effect in buffering the juice.

Although protein is unimportant in buffering the sap it may be a much more important factor in the buffering of the cytoplasm. About 2 % of the whole tuber is protein so that the percentage in the actual cytoplasm is probably much higher than this (at least 5—10 %). By referring to table IX it may be seen that such a concentration of protein would give a quite considerable buffer effect.

The buffer index curve of 0·5 % tuberin is given in figure 23. This percentage in the juice would account for less than 1 % of the buffer indices between pH 4—5 and pH 5—6 and only 5 % between pH 6—7.

VARIETAL VARIATION

A few experiments were carried out to see whether the buffer indices varied from variety to variety. Since the variability in a single variety is so great it is impossible to draw conclusions from such a few observations. These few observations, however, indicate very considerable variation among the varieties.

Five "seed" tubers of each variety were taken and the sap extracted in the usual way. The buffer index determinations were made on the boiled and filtered sap made up to the original volume with distilled water. The results are given in table XI.

Table XI

Variety	Buffer index		
	pH 4—5	pH 5—6	pH 6–7
King Edward VII	0·0160	0·0057	0·0052
The Ally	0·0111	0·0043	0·0031
Up to Date	0·0130	0·0065	0·0036
Arran Chief	0·0157	0·0085	0·0033
Arran Comrade	0·0200	0·0091	0·0059
British Queen	0·0250	0·0090	0·0072

THE INTERACTION OF THE BUFFER SYSTEMS

It might be questioned whether one is justified in assuming that in a given range of pH the buffer index of the sap is equal to the sum of the buffer indices of the separate buffer systems present.

This point was tested in an artificial buffer solution made up to contain citrate, oxalate, phosphate and asparagin. Other solutions were made up containing the single buffer systems in the same concentration as in the mixture. The buffer indices in the various pH ranges were then determined for the mixture and for each of the separate solutions. The results are summarised in table XII.

It is clear that in the main the buffer indices of the mixture are equal to the sums of the buffer indices of the separate buffer solutions in each pH range. The differences between the sum of the indices of the single buffers and the indices of the mixture are all in the same sense. They may, in part, be due to experimental error, but the results of chemical interaction between the

Table XII

Buffer solution	pH range		
	4—5	5—6	6—7
Citrate	0·0070	0·0073	0·0041
Oxalate	0·0042	0·0010	0·0003
Phosphate	0·0008	0·0013	0·0053
Asparagin	0·0008	0·0003	0·0013
Sum	0·0128	0·0099	0·0110
Mixture	0·0113	0·0090	0·0090

buffering substances are not entirely eliminated as a possibility. This work is being continued.[1])

CARBON DIOXIDE EFFECTS

Experiments were carried out to determine the effect on the tuber sap of various concentrations of carbon dioxide. The method used was essentially the same as that described by Martin and is described in Chapter XVII. The results of a typical experiment are given in table XIII.

Table XIII

% CO_2 . .	0	10	20	30	50	70	80	100
Sap pH . .	5·7	5·5	5·3 - 5·4	5·3	5·1	5·1	5·0	4·9

This table shows that the sap in equilibrium with 20 % CO_2 is actually changed in reaction from pH 5·7 to pH 5·4—5·3 and this concentration (20 %) has been recorded by Magness (1920) for the CO_2 in the intercellular spaces of the tuber. A comparison of this table with similar tables given by Martin for the Sunflower sap and for Broad Bean sap shows that CO_2 has a much lower power of changing the pH of the potato sap than it has in reducing the pH of the Sunflower sap but has much the same action on both potato sap and Broad Bean sap. The difference between the action of CO_2 on Sunflower hypocotyl sap and on potato tuber sap is correlated with the difference in the buffering of these two saps.

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[1]) Further investigation, using a quinhydrone electrode, shows an accurate additive effect and the differences found in the above buffer complex compared with the sum of separate buffer systems disappears.

SUMMARY

The use of the "Buffer Index", $\frac{dB}{dpH}$, to express buffer capacity is discussed.

The buffer index of the potato tuber sap increases from an average value of 0·0079 between pH 6—7 to 0·0105 between pH 5—6 and 0·026 between pH 4—5.

The sap of the tuber is much more strongly buffered than that of the leaflets. The buffer index curve of the leaflet and of Sunflower hypocotyl sap are very similar.

Inorganic phosphates in the sap account on the average for 30 % of the buffer index between pH 6—7. In the range pH 5—6 they account for about 8 % and in the range pH 4—5 for less than 1 %.

In a particular case citrate in the boiled and filtered sap accounted for 22·5 % of the buffer index between pH 4 and 5, 39 % in the range pH 5—6 and 19 % between pH 4 and 5.

In this sap sample citrate and phosphate together accounted for 60 % of the buffer index in the range pH 6—7, 47 % in the range pH 5—6 and 19 % in the range pH 4—5.

Substances in the acidified sap soluble in ether are shown to be important buffers especially in the more acid regions (pH 4—5 and pH 5—6) where they account for at least 50 % of the buffer index.

Asparagin and tuberin appear to have a negligible effect in buffering the sap between pH 4 and pH 7.

The buffer index curve of the tuber sap seems to vary greatly from variety to variety.

The interaction of the buffer systems is briefly considered.

In equilibrium with different percentages of CO_2 the pH of the sap varies. In 20 % CO_2, in a particular case, the pH of the sap was reduced from pH 5·7 to pH 5·3—5·4.

3. ADDENDUM

Further investigation of the buffer complex of potato tuber juice by C. T. Ingold has yielded the following data.

Buffer Complex

Important Systems	Inorganic phosphate	0·0053 M
	Citrate	0·0057 M
	Malate	0·00505 M
Subsidiary Systems	Oxalate	0·0008 M
	Asparagin	0·3 %
	Tuberin	0·5 %

Relation to pH

pH range	4—5	5—6	6—7
β for Sap	·0260	·0105	·0079
β for known Buffer Complex	·0092	·0068	·0049

The known buffer complex, therefore, accounts for 35 % between pH 4 and 5, 64 % between pH 5 and 6, and 62 % between pH 6 and 7. Succinic and tartaric acids were not identified in the juice. Treatment of the filtered juice with concentrated tartaric acid gave no evolution of carbon dioxide, so that the bicarbonate-carbonic acid system is apparently absent from the filtered juice.

CHAPTER XV

SUCCULENTS — REACTIONS AND BUFFERS

Succulent plants, with their peculiar metabolism and diurnal variations, have been the subject of much work (see Chapter XI). ASTRUC (1903) made an extensive investigation of the diurnal variations in titratable acids, cp. GUSTAFSON and also LYNN p. 127 HEMPEL's classic work (1917) gives a large collection of data. Her method was as follows — leaves were taken, weighed, and their cell-sap extracted by pounding in a porcelain mortar with a pestle of the same material. The numerous measurements of pH made during these experiments, together with the Buffer Indexes kindly calculated by Mr. C. T. INGOLD from the titration data given by HEMPEL are summarised in Table VIII. The higher pH data were obtained after exposing the plants to light, the lower pH figures after the plant had been in darkness for various periods.

It is obvious that, with the exception of *Aloë arborescens*, all the succulent plants which were compared by HEMPEL after exposure to light and darkness, showed an increase of [H·] under dark conditions. They show also an increase in titratable acid (to the litmus point, pH 6·8) and an increase in buffer index between the natural pH and pH 6·8 (β^1), with the exception of the first two species of *Mesembrianthemum*. The buffer index between pH 6·8 and pH 9·2 (phenol phthalein β^2), shows no such correlation with the changes in natural pH, titratable acid and buffer index in the lower range. HEMPEL from her observations, concluded.

1. That the acidity in *Rochea falcata*, *Cotyledon obvallata* and *C. linguaefolia* "(possibly in succulents generally)" is due not to a mixture of acid and acid salt but to a mixture of acid salt and normal salt, which buffers below the litmus point, i. e. around the natural pH and up to pH 6·8.

Table VIII

Reactions and Buffer Indexes

Plant		Reactions		Buffer indexes		Differences Dark-Light		
		Lakmeid Test	H · electrode	natural pH— to 6·8 β^1	6·8—9·2 β^2	pH	β^1	β^2
Aloë arborescens .	dark	5·0	4·65	·0046	·0017	±	±	±
	light	5·0	4·69	·0046	·0017			
,, cymbaefolia .	dark	4·9	4·69	·0064	·0025	—	+	—
	light	5·4	5·59	·0021	·0030			
Diotostemon . . .	dark	4·4	4·26	·0147	·0134	—	+	—
Hookerii . . .	light	5·4	5·55	·0044	·0161			
Cotyledon linguaefolia	dark	4·2	4·20	·0120	·0047	—	+	+
	light	5·5	5·52	·0055	·0041			
,, coruscans . .	dark	3·9	4·11	·0323	·0048	—	+	+
	light	5·6	5·43	·0072	·0017			
,, obvallata . .	dark	4·3	4·16	·0105	·0047	—	+	+
	light	5·4	5·46	·0081	·0037			
Crassula obovata .	dark	4·1	3·96	·0320	·0125	—	+	—
	light	5·2	4·31	·0263	·0129			
,, lactea. . . .	dark	3·95	3·96	·0247	·0248	—	+	+
	light	5·2	5·13	·0236	·0228			
Rochea falcata . .	dark	4·05	3·95	·0427	·0306	—	+	—
	light	5·5	5·39	·0078	·0391			
Aeonium Haworthii	dark	4·1	4·13	·0327	·0160	—	+	—
	light	4·7	4·49	·0219	·0183			
Mesembrianthemum echinatum. .	dark	4·9	4·51	·0133	·0026	—	—	+
	light	5·6	5·68	·0139	·0025			
,, lingaeforme .	dark	4·8	4·56	·0020	·0023	—	—	+
	light	5·4	5·02	·0032	·0016			
,, Lehmannii .	dark	5·0	4·81	·0077	·0055	—	+	—
	light	5·7	5·35	·0042	·0057			
Kleinia (cuneifolia ?)	dark	4·4	3·97	·0320	·0150	—	+	+
	light	5·8	5·55	·0218	·0057			
Lupinus albus -stalks and first leaves of seedlings		5·9	5·78	·0186	·0823			
			6·03	·0144	·0697			
	19 days. lt.		5·78					
	,, etiol.		5·89					
Lemon juice . . .	—	—	2·19—2·24	(a) ·2183	·0132			
				(b) ·2330	·0025			

1) Reactions as given by Hempel (1917). Buffer Indexes calculated by C. T. Ingold from Hempel's titration data.

2. That the degree of acid formation is not correlated with the degree of succulence „but there is a certain likelihood that it stands in causal relation to the quantity of dissociated malates."

3. That the buffering in the higher pH range is, in part at least, due to precipitation buffering; the alkali precipitating aluminium hydroxide from the aluminium malate present.

4. That in *Rochea falcata* in particular, but also in all the succulents investigated, substances of highly inconstant character occur; these are easily oxidisable and then assume the character of stronger acids.

With reference to the first of these conclusions, HEMPEL herself (p. 65) says "It must be borne in mind that these plants contain not only acids, but also salts of such acids", and further, an inspection of the phosphate titration curve (fig. 11) will show that in the middle region the phosphate is all KH_2PO_4 and K_2HPO_4 within a certain pH range. Similarly within a certain range a malate system will be all acid salt plus normal salt. The pK values for malic acid are given (CLARK 1928, p. 678) as 3·48 and 5·11; so that around pH 5 this conclusion will be correct but at pH 3·95 (see Table VIII) there is a certain small proportion of free malic acid present, as stated in the second quotation.

The second conclusion is in harmony with SPOEHR's view of pentose succulence (1920) and with the findings of DOYLE and CLINCH (1928, p. 119) that there is no really satisfactory correlation between pH and water-soluble pentosan content.

The fourth conclusion is readily explained by SPOEHR's observations (1913) of the decomposition of dilute malic acid in sunlight, giving oxalic (pK 1·42 1st step), glycollic, formic and acetic acids. The disputes concerning the relative proportions in which malic and oxalic acid occur in certain succulents may possibly be due to the same phenomenon, as well as to seasonal variation from oxalate to malate as in *Mesembrianthemum* (see Czapek. III).

HEMPEL (1917, p. 65) also gives a series of curves in relation to the quantities of $\frac{N}{5}$ NaOH required to neutralise 100 grammes of sap, the points being determined with *different* sap samples. The initial [H·] was the natural pH and these are not modern ,titration curves', but by calculating the *apparent* buffer indexes

(Table IX) and comparing these with the buffer index curve of 0·1 M malate (fig. 24 cp. HEMPEL's fig. 15) we can demonstrate diagrammatically the fact that the plants examined form more malic acid at one time than they do at another and that they vary in their capacity for malic acid production. The actual concentration of malates clearly varies from one sample of a particular plant to another.

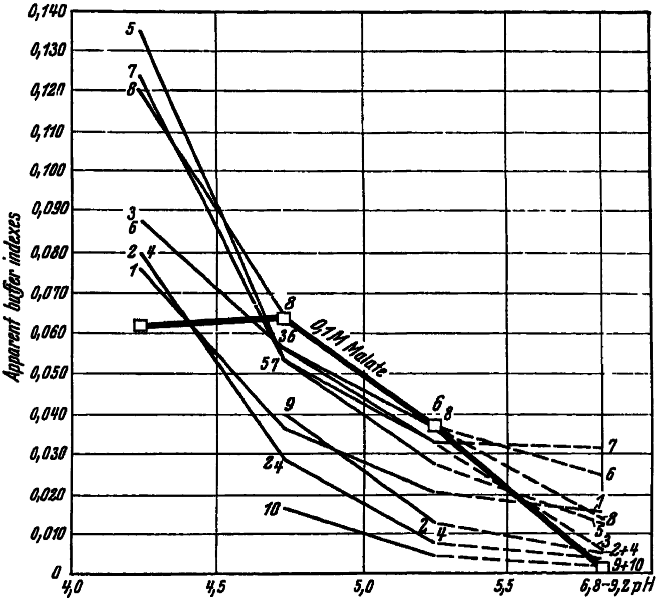

Fig. 24. Apparent Buffer Indexes of Succulents.
(Calculated from Hempel's fig. 11, p. 65. Plants numbered as in Table IX.)

A careful study of fig. 24 shows that, while the decrease in [H˙] from pH 4·5—5·0 to pH 5·0 - 5·5 (except in 2, 4 and 9), might be due almost entirely to change in the acid-malate normal-malate ratio (i. e. to addition of base to the system), this cannot be the case for the zone of lower pH. There the ·1 M malate β value remains constant, the same as for pH 4·5—5·0, and any increase or decrease in [H˙] must be due to the actual formation of more or less malic acid in the plants. If there were an actual production of more acid between the two ranges pH 5·0—5·5 and pH 4·5—5·0, the β curve for ·1 M malate between the points would be flatter than the actual curve found for the plant juice; therefore acidification in this pH region is due to withdrawal of base. While it does not coincide (except with *Kleinia*), the malate curve is almost parallel with the majority of the natural curves, indicating base changes rather than acid changes in the middle zone of pH. Calculating from the β values, in this middle part of the curve, the malate concentration varies from 0·106 M in *Kleinia* to ·046 M in two species of *Cotyledon* (see col. 8 Table IX).

Table IX

Apparent Buffer Indexes

β calculated with some extrapolation from the chart p. 65 in HEMPEL (1917)

	Plant	β in range—pH			β (dark) see p. 293		Molar Conc. of Malate calculated from β
		4·0—4·5	4·5—5·0	5·0—5·5	average below pH 6·8	6·8—9·2	
1.	*Diotostemon*	·076	·036	·020	·0147	·0134	- -
2.	*Cot. obvallata*	·080	·028	·008	·0105	·0047	0·046 M
3.	*C. coruscans*	·088	·056	·032	·0323	·0048	0·093 M
4.	*C. linguaefolia* . . .	·080	·028	·008	·0120	·0047	0·046 M
5.	*Crassula obovata* . .	·136	·052	·028	·0320	·0125	0·086 M
6.	*Cr. lactea*	·088	·056	·036	·0247	·0248	0·093 M
7.	*Rochea*	·124	·052	·032	·0427	·0306	0·086 M
8.	*Kleinia*	·120	·064	·036	·0320	·0150	0·106 M
9.	*Mes. echinatum* . . .	--	·040	012	·0133	·0026	0·066 M
10.	*Mes. lingaeforme* . .	--	·016	·004	·0020	·0023	

The flattening of the slopes in the middle region of *Diotostemon* (1) and for *M. lingaeforme* (10) correspond to the flattening which occurs with lower concentrations of malate. The curves 2, 4 and 9 are steeper than malate curves, indicating actual formation of more acid in these plants within the middle range of variation.

A similar analysis of the rather small scale titration curves, given for *Bryophyllum* juice by GUSTAFSON (1925, fig. 3), shows that there is distinct evidence of malate formation in that case. In the afternoon (3·45 p. m., fig. 3 G) the buffer system is one which falls like a phosphate-bicarbonate buffer complex with some malate present. At midnight (fig. 3 H) the effective buffer system agrees accurately with a ·05 M malate system; while in the morning (fig. 3 F) the effective buffer system appears to be entirely ·073 M malate. GUSTAFSON does not give the actual points between pH 5 and pH 8 for curves F and G, otherwise these suggestions could be checked for the higher pH ranges as well as for the natural pH range of the acid sap. A titration of the sample G with acid as well as with alkali would have shown whether the malate was all used up or remained partly as neutral salt at that time of day. GUSTAFSON's data suggest a nocturnal increase of 50 % in malate content; while HEMPEL's data indicate a doubling or trebling of malate content in the dark, with persistence of a certain proportion as neutral salt even in the light.

The diurnal variations in succulents have already been noted (Chapter XI) and the early work is adequately summarised by HEMPEL (1917), RICHARDS (1915) and others. The more recent work on *Bryophyllum* by GUSTAFSON (1924 b, c, 1925) emphasises the differences in pH which occur in the juice from different parts of the succulent plant and the marked influence exerted by the weather upon both diurnal changes and differences (gradients, etc.) in the actual pH (pp. 124—127). The tissue differentiation found by Miss LYNN (p. 127) emphasises the variation from tissue to tissue in both actual pH and in pH changes, especially with reference to the constantly very acid mucilage cells of the hypodermis and bundle sheath.

The lack of correlation between succulence and pH or even total acidity is in harmony with GUSTAFSON's (1927) remarks upon the concomitance of low pH and rapid growth in the tomato. SPOEHR (1919, 1920) has shown that succulence is associated with a pentose metabolism. The fact of the matter is that while this pentose metabolism is associated with low pH values, the accumulation of acids or acid salts may be either an accumulation of by-products or quite a separate phenomenon. The pentose metabolism may be the cause of succulence and the cause of low pH values at the same time. Rapid growth in the tomato might, therefore, be neither the cause nor the effect of low pH values, but another effect of the same cause — i. e. of an abnormal metabolism.

In the same way low pH values occur, as in Polygonaceae, Rosaceae, *Rhododendron*, etc., without any corresponding succulence, presumably from some other cause than pentose metabolism and therefore unaccompanied by any succulence. Further, succulence may occur (e. g. *Gasteria verrucosa*), also in halophytes (e. g. *Salsola kali* p. 105), associated with relatively high pH values, so that although a pentose metabolism may be one cause of succulence, there may be other causes of the same phenomenon.

Úlehla in a series of contributions (1925—1928, see also Lloyd and Úlehla 1926) on various aspects of desert succulents has given some pH data. The diurnal variation in *Opuntia* has been noted already, p. 128, and other points will be discussed in Chapters XVI and XVIII. In 1925b he gives a number of factors which influence water-intake in plant cells and states that "Any given water factor of the cell as a whole changes in an independent manner under different conditions, such as pH, previous water-content, salts, narcotics, etc." The rest of his relevant data deal with the isoelectric point of plant tissues, see Chapter XVI; and with the interactions of pH and the wall of the living cell, see Chapter XVIII.

Apart from Hempel's pH figures of the juices of succulents and those given in connection with diurnal variation, there are few precise data, except the tissue reactions of the stems and leaves of succulents given in Table VI Chapter X, see all the species marked 'S'. The diurnal variation occurs in the mesophyll only of *Sedum praealtum* leaf, pH 5·6 *ca.* in the light becoming pH 5·2—4.8 in the dark for these tissues, with a variation from cell to cell in the upper epidermis and lower epidermis. An inspection of these records will show that for a species marked 'S' the pH range is usually about c, pH 5·2—4·8. There are, however, notable exceptions; on the alkaline side we have *Aloë variegata* (cp. Hempel on *Aloë arborescens*), *Gasteria verrucosa*, *Salsola kali* and *Veronica beccabunga*; on the more acid side we have *Puya* leaf (pH 4·0 in mesophyll), *Mesembrianthemum stelligerum* leaf and *Crassula rosea* leaf with some cells in the mesophyll at pH 4·0 *ca.*; *Crassula* sp. with mesophyll at pH 4·4—4·0, also *Echeveria* spp. and *Echinocactus* with a more acid upper epidermis. The general occurrence of a pH 5·2—4·8 is of significance in connection with Úlehla's 'Geweberegulation bei Sukkulenten', see Chapter XVI.

BUFFER SYSTEMS IN SUCCULENTS

According to Hempel (1917, pp. 64—65) malic acid is the predominant organic acid present in succulents, not only in Cactaceae but also in Crassulaceae (Aberson and Schmidt cited) and in *Mesembrianthemum* species (Berg and Gerber cited) (e. g. *M. lingaeforme*). Oxalic acid is generally believed to be the chief acid in the last genus but malic acid also occurs in quantity. Mayer (1887) established the presence of isomalic acid in Crassulaceae. Purjevics (cited by Hempel) found acetic and formic acid as decomposition products. Spoehr (1913) found oxalic, glycollic, formic and acetic acids as decomposition products, and oxalic as well as malic acid in *Opuntia versicolor*. Hempel (pp. 16—17) was unable to detect other than minute traces (less than 1 %) of oxalic, tartaric or citric acid in the sap of *Rochea falcata*.

Malic or isomalic acid would, therefore, appear to be the chief acids in succulents and these with their acid salts and normal salts would form the chief buffer systems. One or more of the other acids, however, with their salts may occur as subsidiary buffer systems which might become important in certain pH zones if the buffer index curves of the acids present differed in form and position from that of the malates. Above the litmus point any of the acids mentioned exert practically no buffer effect at all, and in this range (which seldom occurs in any flowering plant, far less in succulents) aluminium might act as a buffer (see Hempel p. 56). In the range of reaction between pH 5·2 and pH 6·2, the higher range found in flowering plants, the organic acids are mainly negligible as buffer-producing substances; and the phosphates and carbonates together with some nitrogen-containing acids suggested by Hempel (p. 62) are the only substances which exert a large buffer effect for a small concentration of buffering substance.

The range of buffer index should be noted. For lemon juice below pH 6·8 it is in the region of normal citric acid (see Fig. 28). In *Aloë* and *Mesembrianthemum* species it is in the same region as in the bean and sunflower sap, while in the other succulents the range of buffer index varies below pH 6·8 from ·0427 to ·0105 (for darkness) and from ·0263 to ·0032 (for light). The higher figures are in the decinormal range of concentration while the lower figures are in the range of centinormal concentration. All these indexes are, of course, lower than maximum on account of the "error of grouping". They are *average* buffer indexes over a wide range.

Thus both qualitatively and quantitatively the buffer-systems in succulents appear to be similar to those which are localised in the *more acid tissues* of normal plants, while the buffer systems characteristic of normal parenchyma occur but are relatively unimportant in succulents. Citric acid, which does not appear to be common in succulents, may be a common buffer-producing substance in ordinary plants.

That the decomposition of acid products in cacti is not an enzymic process appears probable from observations by SPOEHR (1913), who found that the photolytic decomposition of malic acid was accelerated by something in the cactus sap which survives boiling and calcining and precipitation by alcohol and which dialyses out from the alcoholic precipitate. This accelerator must therefore be an inorganic substance and SPOEHR concludes that salts of some kind must be acting. The actual accumulation of the original acid products may, however, be due to enzymic respiration effects, in malate-producing succulents as in the citric-producing fungus *Aspergillus niger* (see CURRIE 1917 and CHALLENGER etc. 1928). The last authors maintain that the citric acid is produced from glucose *via* gluconic acid and saccharic acid.

According to HENDERSON (1913, pp. 223 sqq.), in a solution of glucose there are probably three different forms of glucose which pass freely into one another and ultimately attain a state of equilibrium. Under the influence of a small quantity of alkali further changes occur, resulting in the formation of mannose and levulose, both of which again exhibit three forms in solution. Under these same conditions other changes proceed slowly leading to the formation of lactic acid, methyl-glyoxal, etc. "It is also certain that a great variety of other simple sugars resembling glucose, levulose and mannose are produced, and, all told, the constituents of such a solution probably number at least two hundred, all produced from glucose alone, under the influence of a slight excess of hydroxyl ions." Alkaline hydrolytic and other changes are the types considered here but acid hydrolytic and other changes of sugars are likely to give at least as many possible products. The enzymes present, their relative activities being governed by their optimal pH ranges, would give the bias towards the formation of one kind of product rather than another and it is probably here that we must look for a solution of the various types of acid-producing metabolism, whether these are occurring in succulents or in the more acid tissues of normal plants.

CHAPTER XVI

PROTOPLAST AND pH

1. The real pH of Cytoplasm — (a) errors of methods; (b) pH values recorded; (c) pH values in relation to errors; (d) can the pH be determined? (e) the evidence.
2. pH and the Protoplast — (a) enzyme action and pH; (b) chromosomes; (c) viscosity and pH; (d) staining; (e) permeability; (f) equilibrium points and buffers; (g) membrane buffering.

1. THE REAL pH OF CYTOPLASM

(a) *Errors of Methods.* — A consideration of the errors, described in earlier chapters, for electrometric methods of measuring hydrion concentration, together with the undoubted presence of a large percentage of protein material in the cytoplasm, will make it obvious that the intrinsic protein errors combine with the errors due to protein poisoning of the electrode, thus making all electrometric methods useless for the determination of the pH of cytoplasm. The only attempt at accurate electrometric determination on plant cytoplasm was made by Taylor and Whitaker (1927) on *Nitella* and they got *positive* readings on the galvanometer. Heilbrunn (1928, p. 32) suggests that membrane formation might interfere with the action of a micro-hydrogen-electrode when introduced into the cytoplasm, thus still another error is possible. Electrometric measurements of the real pH of cytoplasm are, therefore, apparently impossible with our present technique.

Further consideration of the previously described errors for indicator methods, in the presence of proteins and other colloids, serves to emphasise the protein error, the lipoid error, the adsorption and chemical change errors and that due to the different dielectric constant of the colloidal medium. Reiss (1926 p. 64) found a difference of 0·5—1·0 in the pH indicated for gelatin fragments and the circumambient medium with various indicators and made quite a legitimate comparison between this system and the cytoplasm-sap system of the cell. Adsorption and differential diffusion in the colloidal cytoplasm destroys more or less completely any possible accuracy of indicator methods when the basis of measurement is the *tint* of any indicator. Protein and lipoid errors may combine to produce quite a deceptive virage when *tints* are used.

There is no evidence, however, that either adsorption or protein error can change the virage of any indicator from the 'alkaline' to the 'acid' ranges used in the R.I.M. Actual changes of the colour of an indicator, especially if supported by other indicators giving a series of similar changes in the same sense below and in the opposite sense above the indicated pH range, can be taken as decisive, for the measurement of cytoplasmic pH. Many indicators are, however, more or less toxic to living cytoplasm and only *intra-vitam* indicators can be used with success for such measurements. Aqueous methyl red, diethyl red and neutral red are such indicators, while brom-cresol green, brom-cresol purple and brom-thymol blue are not rapidly toxic. The indications given by cytoplasm with the first three indicators may be taken with assurance; the value of those given with the second three indicators depends largely upon the technique used. If penetration of these second three indicators into any particular cytoplasm precedes the onset of toxic effects the results may be used in support of the indications given by the vital stains. It must be remembered that cytoplasms differ considerably and that some are much more sensitive to toxic influences than are others. If, on the other hand, the toxic effects begin before the indicators penetrate the actual cytoplasm the virage cannot be taken as that shown by normal living cytoplasm, and this difference may well be a colour difference (cp. SCHAEDE below).

(b) *pH Values recorded.* The old observation by HERRMANN (1879) on the change of red litmus paper to blue by the plasmodium of *Aethalium* might be taken to indicate a pH 6·8 or over for that cytoplasm but the change points of litmus materials may vary. CLARK (1928 p. 86) gives lacmosol as red 4·4—5·5 blue; lacmoid as red 4·4—6·2 blue; azolitmin (litmus) red 4·5—8·3 blue, all cited as SORENSEN 1909 and 1912. According to HEMPEL

(1917 pp. 11—12) lacmoid paper can be used over a range from pH 3·8 *ca.* to pH 6 *ca.*, while litmus paper shows a series of tints from red through violet to blue; one of which tints was used by HEMPEL as the litmus point, namely the particular tint given to litmus paper by a special phosphate buffer mixture at pH 6·81. The "blueing" observation by HERRMANN might, therefore, refer to a tint given by litmus paper at any hydrion concentration from pH 5·5 *ca.* upwards. Further, by means of the R.I.M. it has been shown (p. 95) that the internal pH of fungi is frequently higher than in flowering plants.

One of the most interesting attempts on cytoplasmic pH is that by SCHAEDE (1924). He immersed epidermal cells from onion bulb scales in methyl red solution and in a few minutes found the cell sap red while the protoplasm and the nucleus were yellow. The indicator did not appear to be toxic, since protoplasmic streaming continued and the cells remained plasmolysable. When cells were previously injured the cytoplasm showed a red virage. Similar attempts by M. W. REA and S. H. MARTIN with the same material and various other easily isolated plant tissues failed to give decisive differentiation with indicators and further experiments by C. T. INGOLD with onion epidermis likewise failed to confirm this most interesting record.

The reaction pH $>$ 5·6 for the natural living cytoplasm and nucleus of the onion, given by SCHAEDE's observations. is one of the few records yet published of the hydrion concentration of the cytoplasm as distinct from the rest of the cell. This result rests upon a yellow colouration of the cytoplasm and nucleus with intravital staining by methyl red.

As a case from the animal world, we have the microspectroscopic observations by VLÈS and VELLINGER (1928) on the natural pigment of *Arbacia* eggs. Their conclusion that the pH of the cytoplasm in the neighbourhood of the pigment lies somewhere between pH 5·2 and pH 5·9 seems a very careful statement of the facts, especially in view of the variation in pH found by REISS (1924) using another method on the eggs of *Echinocardium* and *Paracentrotus* both of which varied in the range pH 5·4 to pH 5·65.

A recent contribution by COLLA (1928) in *Protoplasma*, on the action of pH on protoplasmic streaming in *Chara crinita* L., includes almost incidentally a second series of observations of pH in plant cytoplasm (which are not even given amongst the „Conclusions"!). The technique appears to have been very careful and free from many of the usual objections; for example the isoelectric point of the protoplasm was determined directly by MICHAELIS' cataphoresis method, instead of the crude methods recently in vogue (see p. 319). One is, therefore, inclined to give full weight to the following statements (COLLA, p. 187).

„*Reazione del succo cellulare.* Con la porpora di bromocresolo il protoplasma prende una tinta giallo rose che corrisponde a pH 5·4—5·6. I granuli proteici sono colorati in giallo (pH 5·2 [5·00]). Col rosso metile si hamo colorazioni corrispondenti ad un pH press' a poco eguale.

Reazione del vacuolo centrale. Immergendo le alghe in una soluzione al 5 per mille di rosso neutro o rosso congo, fatta con l'acqua in cui esse vivevano, si notarono i vacuoli colorati rispettivamente in rosso papavero od in rosso violaceo (pH 6·7—7·0). Schiacciando il vacuolo in una soluzione diluita di porpora di bromocresolo o di azzurro di bromotimolo, si osserva che gli indicatori assumono una colorazione che corrisponde a pH 6·6—6·8".

Although tints are compared, which is not really legitimate when dealing with protoplasm in particular, we are given some of the facts. From the yellow of B.C.P. we can again say that the cytoplasmic reaction is pH <5·9; and had the colours with methyl red been given we might have obtained a lower limit. The sap reaction is in accordance with that found for several other algae.

The many observations of cells, living and killed made in this Department, have led the writer to the following conclusions —

(1) That when killed or injured the cytoplasm and nucleus may take up the red form of methyl red and show a virage which indicates a pH between 5·2 and 4·8;

(2) That while alive the cytoplasm even of very acid plant cells does not show red with methyl red either because the red form of this indicator does not penetrate or because the reaction is above pH 5·2, but we have only one doubtful record of an actual yellow virage in addition to the potato records, p. 270;

(3) That with diethyl red, a relatively non-toxic and easily penetrating indicator, the living cytoplasm shows red or yellow indicating a variation in reaction from pH 5·6 to pH 5·9, but it has as yet been found with certainty to be yellow when the sap

is red only in the acid cells of potato, although the nucleus of the living cell frequently shows a deeper red than the rest of the cell with this indicator, possibly because of other than pH phenomena;

(4) That while alive the cytoplasm even in less acid plant cells does not show more than a faint mauve with brom-cresol purple and is never, so far as our observations go, green or blue with brom-thymol blue;

(5) That the reaction of plant cytoplasm must lie somewhere between pH 5·2 and pH 6·2 with little or no variation beyond those points, but with probable variations within that range;

(6) That the use of aqueous diethyl red and methyl red, combined with brom-cresol purple and brom-thymol blue used with special attention to toxicity effects, is the best method of determining approximately the pH of any particular cytoplasm under specified conditions. The indications obtained by this method would appear to make the range indicated somewhat narrower than the microspectroscopic method used on natural pigments. The application of the method is open to all, but records should state the precise conditions under which any particular pH is found. There seems little doubt that cytoplasmic reaction does vary within the limits given.

(c) *pH Values in relation to Errors.* All other values given in the literature for cell reactions would appear to be values for vacuolar sap or cell walls or a mixture. The errors given above do not apply to SCHAEDE's observations nor to the R.I.M. The error of mixing sap and cytoplasm applies to all "juice" determinations and to such of the R.I.M. records as are subject to the error of too great an alcoholic concentration. These vary with the indicators used and with the reactions found, and it would take up far too much space if an attempt were made to analyse these records from this point of view. In the main they stand for cell sap and cell wall values, rather than detailed observations on particular cells with a careful differentiation of sap and cytoplasm.

(d) *Can the pH of Cytoplasm be determined?* SCARTH (1924) suggests a possible negative answer, quoting experiments and observations on *Pelomyxa palustris*, a multinucleate amoeba, with neutral red as an indicator, similar to those on *Stylonychia* and *Vorticella* by METCHNIKOFF (1889) with litmus granules.

Vacuoles, cytoplasmic granules and external medium may all show different tints or even different colours. There is nothing in these facts against the possibility of determining the pH of cytoplasm, provided that technique and observation are both careful and provided that the R.I.M. method of interpretation using colours only is followed.

Weightier suggestions are advanced by **Pfeiffer** (1926, 1927) and **Keller** (1928), who emphasise the colloidal nature of cytoplasm. The latter and his collaborators, having specialised for many years with valuable results, upon the electric charges of protoplasm, are naturally inclined to see in these charges the master factor of biological phenomena. Perhaps it is! This monograph on pH in relation to plant cells and tissues is the result of a similar specialist bias on the part of the present writer. **Keller** (1928) points out that —

(1) Capillary chemistry has recognised within the last decade that, in the immediate neighbourhood of membranes, proteins and hydrophilic colloids, which usually have a negative charge of their own, anions are quite motionless and kations have only a strictly limited mobility.

(2) Experimental researches on the permeability of animal and plant plasma-membranes have led to an agreement that strong alkalies and acids do not penetrate living protoplasts at all and strong electrolytes do so only slowly and to small extent. "Die physiologischen Theorien der besonderen Wirkungen von H- und OH-Ionen verlieren durch dieses übereinstimmende Ergebnis verschiedenster Untersucher stark an Wahrscheinlichkeit"! This is the natural result of the wide-spread looseness in locating the various processes, which are supposed to be governed by the pH of the cell, in the cytoplasm itself. There is a distinct possibility that most if not all the processes, in which hydrion concentration plays a conspicuous part, are either surface reactions taking place, not in the cytoplasm but at the surface where cytoplasm meets vacuolar sap, or pH and buffer phenomena of the sap itself. All the detailed observations, including even those of **Scarth** mentioned above, seem to indicate some such arrangement. The work by **Keller** and his collaborators, pushed to its limits with refined technique should prove of great value in clearing up this difference between cytoplasm and sap.

(e) *The Evidence.* All the available data indicate that the cytoplasm, as a colloidal solution of considerable protein content, is strongly buffered not only by its actual composition, but also by the surface precipitation membrane-forming reaction so ably expounded by **Heilbrunn** in Chapters XIII—XIV of Protoplasma Monographien, Band I.

This being so we might expect a relatively stable hydrion-concentration in cytoplasm. All the slight but valid evidence of the actual reaction of plant cytoplasm is derived from observations with the indicators methyl red, diethyl red, brom-cresol purple, brom-thymol blue and neutral red. All these indicators give with various plant sources and with various investigations results which, on an R.I.M. interpretation, agree in indicating a reaction for plant cytoplasm in the range pH 5·2—6·2. **Schaede**'s result gives pH>5·6 (onion); **Colla**'s result gives pH<5·9 (*Chara*); while **Vlès** and **Vellinger** give pH 5·2—5·9 (*Arbacia*). Our R.I.M. results all agree with a range between pH 5·2 and pH 6·2 for living cytoplasm, and pH 5·9 *ca.* for the cytoplasm of potato cells.

2. pH AND THE PROTOPLAST

The actual pH of cytoplasm, from the few pieces of valid evidence which we have, would appear to be distinctly limited in its range, but cytoplasm can undoubtedly exist in the living state in contact with fluids the reaction of which varies over a larger range, pH 2·0 to pH 10 approximately. The important problems, therefore, become the behaviour of cytoplasm and cytoplasmic secretions with relation to hydrion concentration.

The available data concerning these phenomena are for the most part very confused and frequently contradictory, but on one type of cytoplasmic secretion we have a certain amount of more or less precise information.

(a) *Enzyme Action and pH Optima.* The relation of enzyme action to the hydrion concentration of the substrate has been the subject of much work from **Kanitz** (1903), **Senter** (1904/05) and **Fernbach** (1906) up to the classic contributions (1909-onwards) by **Sørensen** and **Michaelis**, **Bunzell** (1916), **Euler** (1920), **Falk** (1921), and a host of others. The references up to 1922 are given in some detail by **Clark** (2nd edition) but the subject up to 1928 is considered in sufficient detail for present

Table X *Optimal Reactions for Plant Enzymes*[1]

Enzyme	Source	Class	pH Optima	Notes
	Aspergillus niger	A	3·5—5·5	
Amylase	„ *oryzae*	A	4·8	saccharogenic test
		A	3·0	viscosity test, 4·0 in buffer soln.
(diastase)	cabbage, carrot and white turnip	B	6·0	
	yellow turnip	B	4·0—7·0	
	Fusarium, Colletotrichum	B	6·0	
	malt	A	4·4—4·5	4·3 at 25° C; 6·0 at 69° C
	Phaseolus	A	4·0—6·0	for starch solution
		B	5·0—5·5	for reducing sugar formation
	plants (general)	(AB)	5·0—5·4	
	potato juice	B	6·0—7·0	
	saliva	B	5·6	with acetate buffer
		B	6·6	with phosphate buffer
				or 6·1—6·2 with acetate and phosphate. 6·9 in chloride and nitrate
Arginase	various	B	7·0	or 9·5—9·8
Autolysis of yeast . . .	yeast	B	6·1	

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[1] Extracted from *Enzymes* by Waksman and Davison, Table on pp. 46—48 and modified from the text. The classification in the third column is by the present writer, J. Small.

Enzyme	Source	Class	pH Optima	Notes
Bacterial enzymes . . .	hemolytic streptococii	C	7·0—7·9	
Bacteriophage (d'Herelle)	bacteria	C	8·0—8·5	
Carboxylase	yeast	B	5·3—6·2	
Catalase	vegetables	B	7·0	4·5 and 8·5 for periods injurious up to 10·0 for short incubations
	bacteria	C	7·5—8·0	range 3·1—9·1
Cellobiase	fungi	B	6·0	
Cytase	various	B	weakly acid	
Desamidase	yeast	C	7·6—8·0	grown on asparagin
Dextrinase	*Aspergillus oryzae*	A	4·0	
Emulsin	—	B	near neutrality	inactivated by acids and destroyed by alkalies
amygdalase.	—	B	6·0	
prunase	—	A	4·4	
β-glucosidase	—	A	4·1—4·4	varies with substrate
Endoenzymes	Pneumococci	C	7·0—7·8	
Erepsin	yeast	C	7·8	
glucosidase	*Asp. oryzae*	B	5·8—6·6	
β-glucosidase	,, ,,	B	about 5·0	
Glycero-phosphatase. . .	,, ,, also seeds yeast, etc.	B	5·4—6·0	
Inulase	various	A	3·8	
Invertase	*Asp. niger*	A	2·5—3·5	
	potato juice	A	4·0—5·0	

Table X. Cont.

Enzyme	Source	Class	pH Optima	Notes
Invertase	yeast	A	4·4—4·6	at 52·1° C. opt. zone 3·5—5·5
	..	A	4·2	at 22·3° C.
	fresh yeast cells	A	4·2—5·2	
	Pneumococcus	B	7·0	
Laccase	*Rhus* spp. and fungi	B	6·7	range 4·5—9·0
Lactase	yeast	B	about 7·0	
	almond	A	4·2—4·6	still active at pH 3·0
Lipase	*Ricinus communis*	A	5·0	
	bacteria	C	7·2—9·0	
Maltase	*Asp. oryzae*	A	4·0	3·0 at 35·5° C.; 7·2 at 47° C.
	bear yeast	B	6·6	
Oxidase	vegetables	C	7·0—10·0	
Pectase	fruits	A	4·3	
Pectinase	various	B	nearly 7·0	
Pepsin	yeast	A	4·0—4·5	
	malt	A	3·7—4·2	1·2—2·2 for stomach.
Peptolytic enzymes . . .	plants	C	6·24—8·5	6·24 for complex polypeptides 8·5 for glycyl-l-leucine
Peroxidase	vegetables	C	7·0—10·0	
	horse radish	A	4·5—4·75	
Phosphatase	seeds	B	6·5	
Phytase	grain	B	5·4—5·5	

Enzyme	Source	Class	pH Optima	Notes
Protease	*Asp. oryzae*	A	5·1	
Protease	malt	A	3·7—4·2	
	papain	A	5·0	
	bacteria	B	6·0—7·2	
Proteolytic enzymes . .	yeast	C	6·7—8·5	
	malt extract	B	5·8	minimal activity 6·8
Raffinase	yeast	A	4·0—5·0	
Tannase	*Asp. niger*	B	slightly acid	injured by excess acid
Trypsin	yeast	C	8·0	7·0 on peptone
Tyrosinase	potato	C	6·5—8·0	range 5·0—11·0
Urease	soy bean	C	about 7·0	7·0—7·2 varying with urea conc.
Zymase	living yeast	A	4·5—5·5	at 28° C. with no nitrogen
		B	4·5—6·5	at 28° C. with yeast water
Zymo-phosphatase . . .	yeast	B	6·2—6·6	

purposes by WAKSMAN and DAVISON (1928). The actual data can be tabulated. The important points are firstly the range of pH through which the enzyme is active and secondly the optimum point (or zone) of pH for the enzyme action. The range is indicated in a number of cases in Table X, and the pH optimum is more often a zone than a definite point. Further the optimal reaction is known to vary with the temperature, see malt amylase and maltase Table X; and also with the substances which are used either to buffer the solution as in salivary amylase or upon which the enzyme is acting as in β-glucosidase of the emulsin system and zymase. The optimal reaction varies also with the change which is used to test the enzymic activity as in the amylase of *Aspergillus oryzae.*

In spite of these variations, a consideration of the data from the point of view of the internal pH of plant cells is quite instructive. Taking the real pH of cytoplasm to be between pH 5·2 and pH 6·2, we can examine the list of optima. Bacterial enzymes in general and a few derived from yeast are the chief cases where an optimum occurs above neutrality; others are oxidase and peroxidase, peptolytic plant enzymes tyrosinase and urease (= Class C). All the other optima are either below pH 7·0 or around neutrality. Of these a large majority have optima in the range pH 5·2—7·0 (Class B) and could, therefore, exert something like their maximum activity within the cytoplasm itself. A third group may be distinguished (as Class A) which have optima below pH 5·2; this includes some amylases, dextrinase, prunase, β-glucosidase, inulase, invertase, almond lactase, *Aspergillus* maltase, pectase, pepsin, horse-radish peroxidase, some proteases, raffinase and possibly zymase in living yeast. A consideration of these Class A enzymes will show at once that most of them act upon substances which occur in the vacuolar sap or in storage cells rather than in the living cytoplasm of plant cells. In the vacuolar sap or in moistened storage regions these enzymes, secreted by the cytoplasm of the same cells or sometimes by the cytoplasm of special cells as in cereals, could obtain a non-cytoplasmic substrate at their particular optimal reactions. Enzymic reactions at the boundary of sap and cytoplasm are a possibility, but the excretion of the enzyme out of the secreting cell into the endosperm or other storage region is a known fact in many cases and a distinct probability in many others.

The known optimal reactions for plant enzymes may thus be grouped as: A-optima for maximal activity in the sap; B-optima for maximal activity in the cytoplasm or sap; C-optima for maximal activity either in an animal substrate or possibly an abnormally alkaline plant substrate. These class C enzymes are, however, capable of acting at or below pH 7·0 in a number of cases, e. g. oxidase, peroxidase, and tyrosinase, so that they may be active although not at their maximum in normal plant cells.

(b) *Chromosomes and pH.* Another aspect of protoplast activity upon which we have comparatively definite and unconfused information is the visibility or otherwise of chromosomes at different stages and at different hydrion concentrations. Kuwada and Sakamura (1926), using the pollen-grain mother cells of *Tradescantia virginica*, have described (a) the distinct appearance of chromosomes in living material at pH 3·8, (b) the swollen and indistinct appearance of these same chromosomes at pH 6·6, and (c) the re-appearance of the same chromosomes as distinct structures when the reaction was brought back to pH 3·8. The changes of reaction were effected by means of various buffer solutions.

These authors suggest that, since the isoelectric point of nucleic acid is about pH 0·7, the chromosomes are always on the alkaline side and become increasingly hydrophilic with increase in pH. They are distinct from pH 2·6 to pH 5·2; but above that point they begin to swell, so that at pH 5·4 they are swollen and at pH 6·7 scarcely to be distinguished. That these phenomena are cytoplasmic and have practically nothing to do with the sap reaction is clearly indicated by a determination recorded of the pressed contents of the anthers, which give red with phenol red and blue with brom-thymol blue, indicating pH 7·0 for the juice.

Sakamura (1927) in a later contribution utilised the ready penetration of carbon dioxide as a means of changing the internal pH of living cells in a reversible fashion. Pure air and air + carbon dioxide was passed alternately through the microscope observation cell. Pollen mother cells of various plants were used and the carbon dioxide of the cells rapidly diffused outwards when air passed through, thus lowering the $[\mathrm{H}^{\cdot}]$, which was raised again by passing carbon dioxide through the observation cell. The cells, being mounted in 5 % or 10 % sugar solution, remained alive and the chromosomes were invisible or almost so under natural conditions, appearing quite clearly when CO_2 was passed over and disappearing again when the CO_2 was removed by a current of pure air. These phenomena were quite reversible and occurred in all plants examined. In *Secale cereale* the internal pH varied from meiosis (pH 5·8—6·0) to the generative nuclear division in young pollen grains (pH 5·2), and the chromosomes were visible without CO_2 transfusion.

The mechanism of the formation and break-up of the chromosomes in karyokinesis is undoubtedly an alteration in colloidal condition. Hydrion concentration controls these phenomena in a marked degree, but experiments with various salts at various pH values show that other factors in addition to pH are important. The original papers should be consulted for the many interesting details.

(c) *Viscosity and pH.* A critical review of this subject is already available in Protoplasma Monographien Band I by L. V. HEILBRUNN, which should be consulted for details and references. Granulation and coagulation on lowering the pH value below neutral point, followed possibly by a decrease in viscosity at still lower pH values, are more or less generally agreed upon but the mechanism of the action may or may not be different in different cases. Increased viscosity and possible coagulation when the pH values are raised above neutral point are also indicated by some evidence, but we may quote HEILBRUNN with assurance.

"It is obvious that our knowledge regarding the action of acid and alkalies on the colloidal properties of protoplasm is far from satisfactory. There is real need for careful and comprehensive experiments on various sorts of material with different acids and alkalies."

(d) *Staining and pH.* This part of the subject might be divided into two sections; 1. differential staining with acid and basic dyes, and 2, vital staining, but the earlier work is admirably summarised by STILES (1924) and a brief historical review will be sufficient (cp. RUNNSTRÖM 1928, p. 237). BETHE (1916, 1922) produced experimental evidence for the general rule that relatively alkaline cells take up basic dyes while relatively acid cells take up acid dyes. ROHDE (1917), one of BETHE's pupils, gave evidence which is more or less corroborative. He found that acid dyes coloured acid cells (pH 3·09—5·5) very quickly and intensely, also that basic dyes coloured these cells slowly but distinctly.

Rohde also found that neutral tissues in acetate buffer (at pH 5·66—4·14) became acid in their response to acid dyes, while acid tissues with phosphate buffer (at pH 8·12) changed to relative alkalinity in their response to basic dyes. There is, therefore, no doubt that the pH, both internal and external, affects in some degree the capacity of living cells to absorb acid and basic dyes. The effective internal acidity in this case appears to be that of the sap.

Irwin (1923) found diffusion of cresyl blue into *Nitella* cells very slow below pH 5·9 and increasing from pH 5·9 to pH 9·0. Irwin (1928) using *Valonia* found methylene blue penetration at pH 5·5 and pH 9·5 so slow as to be not easily measurable.

Sideris (1925) using *Fusaria* spp. distinguished two kinds of pigments — (a) diffusible and escaping from the cells, (b) non-diffusible and retained within the cell. He found that the initial pH of the medium always controlled the colour of the pigment and that, only if the initial pH were maintained, it controlled also the initiation or inhibition of pigmentation.

Since [H˙] is intimately connected with rH, all the new studies of oxidation-reduction potential are carried out on material at the same pH. This sameness may be natural as in the two [H˙] kinds of spores of *Equisetum* (Joyet Lavergne 1928, see also Needham 1926), or it may be obtained by buffer control of the external medium combined with control of the internal pH by means of CO_2 and NH_3, as was done by Brooks (1926). Brooks (1927) maintained that methylene blue penetrates as such into *Valonia* and that the rate of penetration depends upon the pH of the external fluid and the temperature. Irwin (1928) disagrees, see above.

Quite a number of important contributions bearing upon the problems of vital staining in relation to hydrion concentration have appeared in *Protoplasma*. Rumjantzew and Kedrowsky (1926) deal with protista; Needham (1926) with oxidation-reduction potential; Küster (1926) is sceptical of vital staining of the protoplast in any case, but did not use methyl red and diethyl red. According to Gellhorn (1927), vital staining depends upon two factors —

1. the reaction of the cell (in Bethe's sense) and —
2. the permeability of the colloidal cell membrane.

He gives data for the eggs of *Strongylocentrotus*, which confirm BETHE's theory. SCARTH (1926) obtained true vital staining, with selective staining of the nucleus by eosin, when *Spirogyra* cells were rendered artificially more permeable by means of strongly hypertonic sugar solution. GICKLHORN (1927) records similar results without sugar solution. PFEIFFER (1927) emphasises the importance of the electric charge of the membrane when dealing with dissociated dyes. COLLA (1928) gives further results with indicator dyes. ALBACH (1928) reviews the subject of vital staining in general and gives new data, concluding that (1) the internal pH of the cell has an influence on the speed but not on the final intensity of the dye-absorption; and (2) the pH of the external medium is the main factor for both speed and final concentration of dye-absorption.

SCHAEDE (1927), having determined the natural degree of acidity in various living plant materials, finds some correlation between the natural pH and the pH of various fixing solutions which give different results with these materials. For example, he finds JUEL and alcohol-osmic-chrom-acetic fixatives (pH 3·0—3·5) useful with the acid onion roots or slightly acid staminal hairs of *Tradescantia* but more or less useless with the neutral or slightly basic roots of *Vicia faba* and *Hyacinthus romanus* which require fixatives of pH 1·1—2·4. NAYLOR (1926) found that washing killed material in buffer solutions of various pH values had a strong effect upon the staining properties. ZIRKLE (1928) gives details of the effect of pH in chromium staining.

(c) *Permeability and pH.* Permeability data in general are dealt with more or less critically by STILES (1924). According to STILES, the only pH observations which are not open to serious objections are those by TRÖNDLE (1920) who found that immersion of leaf cells, of *Buxus sempervirens* for five minutes in 0·005 N HCl or 0·01 N oxalic acid, increased the permeability of these cells to sodium chloride.

WEBER (1926) gives a literature list of permeability papers since 1922, supplementing the bibliography given by STILES (1924). ILJIN (1928) notes briefly (pp. 586—587) the work of some earlier authors but there is practically nothing of critical value apart from the already-mentioned work on dyes. ILJIN finds, using onion, rhubarb and potato tissues, that the permeability varies with the time of immersion either in water plus carbohydrates or in salt solution plus carbohydrates. The method used was quantitative determination of the concentration of the substances per unit dry weight of the tissues, after immersion for a known time in the experimental solutions. Salts of various kinds increase the permeability for inulin, fructose, saccharose, glucose; and the variation for potassium permeability is also given. Other conditions being the same, the permeability decreases with longer immersion. On the action of pH ILJIN is quite precise and quantitative. The effect of the phosphate buffers used is differentiated, as well as the effect of the potassium and sodium ions in the buffers; and the final as well as the initial pH was observed. This part of the work was mainly upon *outflow* of monosaccharides, disaccharides and potassium from the onion; but the sugar content, external and internal, of preparations of *Valonia*, the osmotic pressure (NaCl) of *Chaetomorpha* and epidermal cells of *Rhoeo discolor* were also observed. ILJIN concludes that the pH of the external solution has a great influence on the permeability of the protoplast. In general, the degree of permeability is lowest about the neutral point, increasing with increase of $[H^{\cdot}]$ and also with decrease of $[H^{\cdot}]$. The minimum varies in its position on the pH scale with the ions and molecules permeating.

His data for outflow of sugar yield minima for external pH in different experiments, as follows pH 5·9, 6·6, 7·3, 6·8, 6·9, 7·2—6.9, 5·9. His table for outward diffusion of potassium gives a sharp minimum at pH 5·6. SCARTH's (1926) observations on increased permeability of *Spirogyra* to eosin after immersion in glycerol or in hypertonic sugar solutions do not, of course, affect the validity of these outward diffusion data.

HOAGLAND and DAVIS (1923) used *Nitella clavata* and found the $[H^{\cdot}]$ of the sap usually around pH 5·2, with occasional variations in the range pH 4·8—5·8. They found, using NaOH and HCl for adjustments, that an external variation of the pH in the range pH 5·0—9·4 gave no change from pH 5·2 ±·1 in the sap within the cell, which was very slightly buffered as a solution. Variations of the external $[H^{\cdot}]$ to pH 4·4—3·8 gave slight to decided injury, with accompanying loss of chloride. Further, an external pH 5·0—5·2 gave a large inward penetration, pH 6·2 distinct, pH 7·0—7·2 slight and pH 8·5—9·0 practically no penetration of *nitrate*.

These critical observations give confirmation to MAC DOUGAL's (1926) notes on young cells of *Carnegia gigantea* which show increased permeability and diminished water-capacity in acid and alkaline solutions; to GARNER's (1924) observations on the relation of photo-periodic growth to the pH of the cell sap. A number of miscellaneous observations might be explained along the same lines, e. g. IRWIN on *Nitella* and dyes (1922, 1923); LILIENSTERN (1927) on Saprolegniaceae optimal growth and development of oogonia; PETRI (1926) on the killing of *Ustilago* spores; seed germination phenomena (see STILES 1927).

The markedly selective permeability of the cytoplasm to carbon dioxide and ammonia has been demonstrated in detail by JACOBS (1920—1922) and these results have been used by other workers (SAKAMURA, BROOKS etc.) in controlling the intracellular pH of living tissues. One of the most noteworthy results is that, while the cytoplasm is shown to be relatively impermeable to strong acids [cp. BETHE (1907), HARVEY (1911, 1923), CROZIER (1916), HOAGLAND and DAVIS (1923), KELLER (1928) and WERTHEIMER below], an external alkaline mixture of CO_2 and $NaHCO_3$ *raises* the intracellular acidity, because the $NaHCO_3$ penetrates so much more slowly than the carbon dioxide. A solution of lower pH due to HCl has not such a strong acidifying effect as this alkaline mixture. These new data give greater significance to the older consideration of the carbon dioxide factor by LILLIE (1909), see Chapter XVII.

Although carried out with an animal membrane (frog skin), the observations of WERTHEIMER (1927) are of considerable importance for plant physiologists. Taking the premeability at pH 7·0 as a standard, he finds that it varies with the pH and with the direction of the permeation flow and with the substance permeating. If + = greater, — = lesser and () = unaltered permeability the results may be tabulated thus —

	Inner		Outer	Outer		Inner
	reaction of the inner side			reaction of the outer side		
	pH 6·0	7·0	9·0	pH 6·0	7·0	9·0
NaCl	()		()			-
Na_2HPO_4	+		()	-		()
Traubenzucker	+		—	()		()
Glykokoll	+		+	—		+
	6·0	7·0	9·0	6·0	7·0	9·0

From these data it is clear that, when problems involving the relation of permeability and pH are concerned, (a) any possibility may theoretically be present, and (b) there is nothing to be done but determine experimentally the actual relation under the given conditions (cp. IRWIN 1926c, for cresyl blue with *Nitella* and *Valonia*). The pH effects may or may not be present, and if present they may or may not be effective in controlling the other phenomena the explanation of which is being sought.

WERTHEIMER (ibid. p. 620) also gives a list of organic acids in the order of their degree of penetration with carbon dioxide at the top and lactic acid at the bottom; with tartaric, citric, oxalic, sulphuric and hydrochloric acid labelled "impermeabel". He also gives ammonia and trimethylamine as permeating, dimethylamine slightly permeable, NaOH impermeable. Concentrations over N/100, especially for the stronger acids, were found to be injurious. It does not follow, however, that when a membrane is impermeable to acids it is also impermeable to more or less neutral, dissociated or undissociated salts of these acids (cp. IRWIN 1926a, b, c, PFEIFFER 1927 and KELLER 1928). This becomes important when dealing with the movements of buffering substances. The permeability to weak bases has been dealt with recently by POIJÄRVI (1928), but the natural applications seem very limited.

(f) *Equilibrium Points and Buffers.* At one time a critical and detailed review of the literature on so-called "isoelectric" points in plant cells and tissues was proposed for this section, and notes were taken of the observations and conclusions of about two dozen authors; but, in view of the facts concerning buffer action and its influence, this has been abandoned. These points may or may not be "isoelectric"; further enquiry may elucidate this; but the one clear determination is that given by COLLA (1928) using cytoplasmic granules and MICHAELIS' cataphoresis method microscopically. He records for *Chara crinita* an isoelectric zone ranging from pH 5·4 to pH 6·2 for the cytoplasm, with the actual pH of the vacuolar sap at pH 6·6—7·0, the cytoplasm at pH 5·4—5·6 and the contained protein granules at pH 6·2—5·0.

All the other "points" recorded would be better described as "equilibrium points". The basic contribution is that by YOUDEN and DENNY (1926), followed by an additional paper (DENNY and YOUDEN 1927). These authors do not deny the possible existence of real isoelectric points, but they give conclusive evidence that, in the case of potato tissue, substances come out of the tissue into the immersion fluid and that these soluble substances, which are not proteins, are capable of exerting in watery solution 95—97 % of the buffering effect observed when the tissue is placed in external buffer solutions of various acid pH values, and 75 % of the effect in solutions of various alkaline pH values. The final value to which the external fluid is brought is usually around the actual pH of a watery extract of the tissue. These equilibrium points were for slices of apple (pH 3·45) and potato tuber (pH 6·25—6·4); for potato roots (pH 6·4) and barley roots (pH 7·2); for corn (pH 5 or 6·3), wheat (pH 6·4) and rye seeds (pH 6·25); and for the matted hyphae of *Monilia sitophilia* (pH 5·5).

The demonstration of this phenomenon is proof of what might have been expected, since the addition of a buffer solution to a solution of different pH shifts the pH of the mixture in the direction of that of the added buffer solution. A weak acid and salt mixture behaves, in fact, like a plant tissue with an isoelectric point at the pH of the acid-salt mixture. When 75—97 % of the effect can be shown to be due to soluble buffering substances, the attribution of anything but a subsidiary significance to proteins, cytoplasm or wall, is not justified by the facts.

ÚLEHLA (1928) claims to have established for *Opuntia* tissue a buffer effect which is independent of any substance leaching out from the tissue. A close analysis of the actual data shows that: —

1. With external fluids below pH 5·0 (HCl) both time relations and final pH (32 hours) are almost the same for normal living tissue and tissue killed by heat, chloroform or alcohol.
2. With external fluids above pH 6·0 (NaOH) the time relations are different but the end point (32 hours) is much the same for normal living tissue and tissue killed by heating or chloroform. The alcohol curve shows a different end point (32 hours) but this should be compared with the graph for outward diffusion in alcohol from *Pelargonium*, another acid tissue (see above, p. 56).
3. The imbibition curves (ÚLEHLA, p. 497) clearly indicate injury below pH 3 and above pH 9.8 giving results (at pH 1 and pH 12·5) very similar to those of tissue killed by chloroform.

Such data all show that imbibition is a phenomenon of living tissue while the buffer effect is mainly a non-vital phenomenon.

4. On p. 481 details are given which show that buffering substances do not leach out of *Opuntia* tissue into **water,** other than carbon dioxide which is boiled off before a reaction between pH 7 and pH 8 is found for the immersion fluids. This it taken by ÚLEHLA as proving that malic acid or malates do not diffuse outwards from the same tissue in contact with various concentrations of HCl and NaOH, but if there is anything in the observations recorded in the previous section of this chapter (p. 318) there is still a distinct possibility of exosmosis of buffers under ÚLEHLA's experimental conditions. ÚLEHLA, therefore, does not prove his contention and the internal evidence is all in favour of an outward diffusion of buffering substances into his experimental immersion fluids. The point could easily be settled by taking a titration curve of the external immersion fluids before and after the tissue has been soaked and constructing two buffer index curves. Similar buffer indexes with the tissue removed from the fluid, would support ÚLEHLA's contention satisfactorily; but a rise in the buffer index of the *fluid* after immersion of the tissue would support YOUDEN and DENNY; while a comparison of the second curve with a buffer index curve of the fluid plus tissue in the same volume of immersion solution would establish quantitatively the relative importance of sap buffer-systems and other buffer-systems in the effects observed.

This apparent "isoelectric" effect of any soluble buffer-system and the changes in external pH induced by outward diffusion of buffers, not only leaves much of the work on "isoelectric points" of plant tissues under suspicion but it would also seem to involve the earlier work of STILES and JORGENSEN (1915) and HIND (1916) (see STILES 1924, p. 168 and this volume p. 338), where the reduction of $[H^{\cdot}]$ in the external fluid was taken as evidence of absorption of hydrogen ions by the immersed tissue. The possible outward diffusion of buffering substances confuses all these issues, until it is quantitatively determined, as was done by YOUDEN and DENNY.

The "isoelectric point" records may be treated as **equilibrium points.** When an observed process or phenomenon A is controlled by two factors y and z, and when these two factors y and z are governed by the hydrion concentration in opposite directions, so that the curve relating **y** to pH cuts the curve relating **z** to pH in the form of an X, then the observed process or phenomenon A will reach an **equilibrium point** (maximum or minimum) at the point on the pH scale where the **y** and **z** curves intersect. Where there are more than two factors concerned the maximal or minimal regions will tend to become broader zones on the pH scale, instead of a more or less definite point.

A good example of this is found in the formation of two or three kinds of oxalate salts and two or three forms of crystals of calcium oxalate at various pH values (see p. 100 and PFEIFFER 1925b).

In each record of an equilibrium point, the determination of the factors **y** and **z** and perhaps others has still to be carried out; even YOUDEN and DENNY fail to analyse quantitatively the buffer systems present, as has been done by MARTIN, INGOLD and ARMSTRONG (q. v.).

It may be useful to point out here that organic acids tend to increase in buffer capacity below pH 5·0, while phosphate and bicarbonate systems tend to increase in buffer capacity above pH 6·0. BOTH types of buffer systems usually reach a minimum between pH 5·0 and pH 6·0. A factor which tends to increase the [H˙] below pH 5·0 is met not only by a rapidly increasing buffer capacity of the system already present but may in itself tend to increase the β value. Similarly a factor which tends to decrease the [H˙] above pH 6·0 is met by an increasing buffer capacity of the carbonic acid — bicarbonate system usually present and possibly also of the phosphate system which is commonly present. The result is a natural tendency for these equilibrium points to occur in the valley of the buffer index curve for the systems present, usually in or near the [H˙] range pH 5·0—6·0. The natural pH of normal plant cells is also in this range, with exceptions due to change in the metabolism on both acid and alkaline sides. ÚLEHLA's equilibrium point with HCl and NaOH and an organic acid which tends to break up in light is naturally around pH 5·6.

Concerning proteins and their isoelectric points — these have been determined for proteins as isolated (see LOEB 1922 and others such as OSBORNE, PAULI and ROBERTSON); but as VLÈS (1925, see also REISS 1926) points out, in the living cell the proteins, by reason of their ampholytic properties, are almost sure to form complexes, with the possibility of a large variety of complex proteins which split up into simpler substances during the manipulation for isolation. This variety of protein complexes might exhibit a large number of partial equilibrium points (cp. Strugger, Robbins and others) and would tend to show zones of "isoelectricity" rather than points (cp. Colla, Kopaczewski 1926 b and others). Finally many of the so-called isoelectric point data deal with the destruction of these colloidal protein complexes and the consequential death of the cell, cp. Pearsall and Ewing (1924, 1927), Susaeta (1928) for *Carcinus* sperms, and others. Whether an equilibrium point of any kind is involved or not in these cases still seems doubtful.

Kopaczewski (1926, p. 246) appears to have been correct when he wrote "Pour toutes ces raisons, il faut considèrer la conception de Loeb comme une simplification dangereuse, il faut empêcher les chercheurs d'engager leurs recherches dans cette voie; la somme d'énergie qu'ils consacreront à ce sujet sera en grande partie perdue"[1].

(g) *Membrane Buffering.* Although the external buffering effects of tissue may be largely explicable by means of diffusing buffer substances, there are other aspects which must be considered — firstly the apparently very stable reaction of the cytoplasmic layer; secondly the fact that acid sap content is of frequent occurrence, combined in some cases with a much less acid cytoplasm (see Lundegardh 1922, MacDougal 1926 and Úlehla 1928 for cactus cells, also Flury 1927 for stinging hairs of *Urtica* containing acetic, butyric, formic and other acids, also Chapter X and many other references); and thirdly the fact that strong acids do not penetrate readily into the cell (see Jacobs 1920—1922, Smith 1923, Lapicque 1922, Hoagland and Davis 1923, also many others). All these facts indicate that the cytoplasmic layer in living cells may exert a strong control over the pH of (1) itself, (2) the outward passage of acids and (3) the inward passage of acids.

The cytoplasmic layer, containing as it does a relatively high concentration of proteins and also possibly of the ampholytic phosphatides all in a colloidal condition, is sure to have a marked effect upon the movement of ions of both kinds. This is emphasised by Pfeiffer (1927) and Keller (1928), and explains the high buffer capacity of the cytoplasm so far as its own internal reaction is concerned.

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[1] For a detailed criticism see Kopaczewski 1926b.

The passage of acid or basic substances through this layer, either outwards or inwards, must be governed by the conditions within the layer. Whether these conditions are as postulated in the filtration, lipoid, mosaic, adsorption or electrocapillary theories of permeability, the buffer effect remains.

Moore, Roaf and Webster (1912) suggested that the non-penetration of substances is not due to the structure but to the colloid contained in the membrane. This view receives considerable support from the close similarity in buffering and other properties of an artificial cell containing cholesterol dissolved in lecithin to the extent of 0·1 % to 1 % of the lecithin-cholesterol component (MacDougal and Moravek 1927). Gelatin and agar formed the basis of this protein-phosphatide colloidal membrane. Studies were made of its permeability to various ions and molecules in relation to pH, and maximal or minimal points are recorded at various pH values, e. g. 2·9, 4·5, 4·65, 5·4, 6·5, 7·25 and 7·3. The internal pH was found to remain almost unaltered while that of the immersion fluid varied from pH 3·05 to pH 8·2, so that an undoubted controlling action was exerted by the membrane on the pH of the internal fluid in relation to external changes (cp. Lapicque for *Spirogyra*, also Hoagland and Davis 1923 for *Nitella*).

In addition to the suggested ampholytic buffer effects of the phosphatides, there are the buffer effects of electrocapillary phenomena, all affected by the pH as it reacts upon the hydration capacity and the density of the charge on the colloidal substrate.

Under natural conditions in the living cell this membrane buffering would appear to control the internal pH of the cytoplasmic layer and the inward or outward passage of acids or alkalies as such but not as salts, and it must be remembered that this particular buffer effect is probably limited to the cytoplasmic layer, and that the reaction of external fluids or internal vacuolar fluids is governed by other factors. Finally it should be noted that carbon dioxide and some of the weak acids as well as ammonia and one or two weak alkalies pass readily through the cytoplasmic layer and are capable of altering to quite a considerable degree the reaction of internal or external fluids.

CHAPTER XVII

CELL SAP AND pH

1. The real pH of Cell Sap. — (a) errors of methods; (b) pH values recorded; (c) pH values in relation to errors; (d) can the real pH be determined? (e) the evidence.
2. pH and Cell Sap. — (a) enzyme action and pH optima; (b) carbon dioxide effects; (c) acid-producing metabolism; (d) oxalate crystals; (e) colloids in sap; (f) proteins in sap; (g) equilibrium points and buffers; (h) membrane buffering, sap and cytoplasm.

1. THE REAL pH OF CELL SAP

Unlike the body fluids of animals, the cell sap of plants can rarely be obtained free, unmixed and in quantity but, unlike the cytoplasm, the cell sap is not completely dominated by colloidal phenomena and there is not the same lack of critical evidence in this case.

(a) *Errors of Methods.* These have been dealt with in Part II of this monograph and it is only necessary here to emphasise the importance in connection with cell sap of two errors —

1. The carbon dioxide error of hydrogen-electrode determinations, which renders most of the records obtained by these methods open to serious objection and leaves them as records of more or less residual pH values, with the important carbon dioxide factor more or less neglected according to the degree of precaution used. This also applies in some degree to certain colorimetric determinations, especially those with expressed sap.
2. The error of mixing, which applies to all cases where sap has been expressed from organs in which there are differentiated tissues with possibly different saps. Expressed sap from uniform tissues, when it has been centrifuged or filtered quickly can be taken with due precaution as approximating to the original cell sap. The possible adsorption of buffer substances by the broken tissues may make a difference of some signi-

ficance between the pH of expressed sap and the original pH; cp. **Hoagland** and **Davis** (1923), also **Dixon** and **Atkins** (1913), see p. 276 in Chapter XIV above. The serious aspect of this error, however, lies in the actual mixing of different saps and the mixing of cytoplasmic substances with the cell sap even in centrifuged or filtered juices.

The various other errors detailed previously may become important in special cases.

(b) *pH Values recorded.* **Bennet, Anderssen** and **Milad** (1927) using a suction method in which the carbon dioxide factor could be adequately controlled, found that the 'free' tracheal sap when allowed to reach equilibrium with the air varied with the soil in which the tree grew — clearly a buffer effect with carbon dioxide an effective factor. The values for this residual pH ranged from pH 5·5 to pH 6·4 on acid soils, and from pH 7·0 to pH 7·2 on soils rich in lime. On expelling (the remainder of) the carbon dioxide the [H˙] fell to pH 8·0. The reaction of the sap while still in the vessels was not determined but "is probably kept somewhat acid, even when carbonates (? bicarbonates) are present by the relatively high CO_2 concentration present".

Foà (1906) recorded pH 5·7 for the latex of *Ficus elastica* another 'free' fluid, also pH 4·24 for juice of nearly ripe pear and pH 4·52 for ripe grape juice. **Clark** and **Lubs** (1917) recorded the following values for food-fluids —

Substance	pH	
	raw	Autoclaved
vinegar	2·36—3·21	
beer-wort	4·91—8·55	
maple syrup	6·75—6·8	
silage juice	3·70—3·91	
apple juice (1)	3·76—5·65	3·8
prune juice	4·12—9·44	4·3
carrot juice	5·21—9·27	
cucumber juice	5·08	5·1
apple juice (2)	5·02	
string bean juice	5·23—8·63	5·2
banana juice	4·62	4·6
potato juice	6·06—9·44	6·1
sweet potato juice	5·80—8·73	
beet juice	6·07—8·75	

Commenting on these results McClendon and Sharp (1919) say that the juices were apparently kept for long periods and became more alkaline possibly by fermentation changes, a suggestion which is supported by the records for autoclaved juices. It will be noted that the first values (for fresh juices ?) all lie within the range pH 3·70 for silage juice to pH 6·07 for beet juice. This agrees with the R. I. M. determinations given in previous chapters, where the values for sap are rarely above pH 6·2 and rarely below pH 3·4.

McClendon and Sharp (1919) give their own records for food juices as follows —

Substance	pH	
	raw	after boiling
young carrot juice	5·85	5·80
potato juice	5·57	—
cabbage juice	5·90	5·78
orange juice	3·55	3·55
lemon juice	2·32	2·30

They also give the values already quoted for Clark and Lubs, and cite a list selected by these authors from the literature as follows —

Substance	pH
flour extract	6·0—6·5
beer	3·9—4·7
wine	2·8—3·8
lime juice	1·7
lemon juice	2·2
cherry juice	2·5
grape fruit juice	3·0—3·3
orange fruit juice	3·1—4·1
rhubarb juice	3·1
strawberry juice	3·4
pineapple juice	3·4—4·1
tomato juice	4·2
plant cell sap juice	5·3—5·8

From these data it will be seen that the natural fresh expressed juice of plants rarely shows a $[H^{\cdot}]$ above pH 6·2, and that a metabolism resulting in the production of large quantities of organic acids as in many fruits produces a [H'] below pH 5·0.

This and all the valid records given in Chapters X—XIV gives us a comparatively solid foundation for the general statement that normal plant cell sap has a reaction in the range pH 5·0 to pH 6·2, while disturbance of the normal metabolism usually results in greater production of organic acids giving values which may reach the high [H'] of pH 1·7 as in lime juice.

(c) *pH Values in relation to Errors.* Amongst the records given in Chapters X—XI reactions above pH 6·2 are not uncommon. Practically all these high values were obtained by means of hydrogen-electrode methods on juices which had been partially or completely freed of carbon dioxide. All such records can be regarded only as residual values and of little or no interest when the reaction of natural cell sap is being considered. It should also be noted that records between pH 4·8 and pH 6·2 are also open to objection, if the hydrogen-electrode method has been used, since the carbon dioxide factor may be effective throughout that range mainly as free carbonic acid (especially below pH 5·2) but also as part of a buffer system in accordance with the fundamental dissociation ratio.

(d) *Can the real pH be determined?* The two errors described above are so important that unless they are avoided the real pH of cell sap cannot be determined with any certainty or with accuracy. In the case of free sap the carbon dioxide error has still to be avoided (cp. BENNETT above), and the value pH 5·2 for free *Nitella* sap is only more or less reliable (HOAGLAND and DAVIS). The various errors of indicator methods applied to living tissues render an R.I.M. interpretation necessary if the results are to be considered as real pH values. Using this R.I.M. interpretation, colour data given by ROHDE, ATKINS and others can be rendered more or less reliable. The R.I.M. data may be considered the most reliable and most accurate data yet available but they are open to several objections. Firstly the cutting of the sections may either stimulate the internal production of carbon dioxide or liberate the intercellular carbon dioxide thus changing the equilibrium in the whole system and increasing or decreasing the [H'] (see p. 271). Secondly immersion in the indicator solutions may alter the permeability to carbon dioxide, again possibly changing the pH. Thirdly there may have been a certain mixture of saps of adjacent cells by diffusion when strongly alcoholic indicators were used. Fourthly there may have been some toxic effect in the sections immersed overnight before examination.

Considering all these factors we must come to the conclusion that the real pH of cell sap in any particular case has still to be determined, but at the same time the carbon dioxide factor cannot be very effective below pH 4·4 in the presence of organic-acid-salt buffering; and the lower values in particular, recorded by various methods, can be taken as reasonably accurate. Between pH 4·8 and pH 6·2 the variations and differentations found, using the R.I.M. can be taken as *relatively* true. The actual values (see Clapham, Lynn, Armstrong and Chapter XIV) obtained by the improved R.I.M. can be taken as very near to the real pH, disturbed only by possible errors due to section of the materials which may be observed and taken into account as in the potato (Chapter XIV).

(c) *The Evidence.* The evidence for the conclusions in the previous section involves the bulk of this monograph; since cytoplasmic pH is almost negligible in the literature and the special data for the cell wall are considered only in the following chapter. The reader may have realised before reaching this point that the reaction of plant cells and tissues is mainly the reaction of the cell sap. It should be unnecessary further to emphasise this here.

2. pH AND CELL SAP

In dealing with the sap the factors and phenomena are those of free solutions with colloidal and membrane effects more or less subsidiary.

(a) *Enzyme Action and pH Optima.* These are given in Chapter XVI, and attention has already been directed to the Class A enzymes as acting upon substances such as sugar or inulin which occur in the sap, and storage starch or protein which occurs in the cavities of more or less dead cells. These enzymes have optima below pH 5·2, while a large majority of the other enzymes have optima between pH 5·2 and pH 6·2 which is the normal range for sap as well as the apparently constant range for cytoplasm.

(b) *Carbon Dioxide Effects.* Carbon dioxide can cause a reversible liquefaction or coagulation of cytoplasm and readily permeates through both plant and animal cytoplasm (see JACOBS 1920—1922 and HEILBRUNN 1928 pp. 185—187). These phenomena, combined with the metabolic production and utilisation of carbon dioxide in plants, place carbonic acid in a very important position in relation to the phenomena of hydrion concentration especially in the range pH 4·8 to pH 7·0, i. e. throughout the normal range of sap and cytoplasm reaction and higher on the alkaline side. The effectiveness of this carbon dioxide control of the pH of sap is considerably increased by the occurrence between pH 5 and pH 6·2 of a region of minimal buffer action in the organic-acid plus phosphate-bicarbonate complex of buffer systems.

Theoretical. — Our knowledge of buffer complexes in plants is as yet very meagre, but if one looks through a list of pK values for buffer capacity maxima (e. g. CLARK 1928 p. 678) one finds the following pK values — azelaic 2nd 5·6, *citric 3rd 5·49*, *glutaric 2nd 5·54*, itaconic 2nd 5·7, *1-malic 2nd 5·11*, *malonic 2nd 5·68*, o-phthalic 2nd 5·41, *pimelic 2nd 5·41*, pyrotartaric 5·63, sebacic 2nd 5·6, *succinic 2nd 5·57*, sulphurous 2nd 5·3, uric 5·8, *histidine 5·66*. All the other acids, including the amino acids, have their maximal buffer indexes below pH 5·0 or above pH 6·0. Of the acids mentioned above glutaric, malonic, pimelic and histidine have been found but seldom in plants. The others are not known to occur in plants (cp. CZAPEK 1925 and EULER (1908) with the exception of the *commoner acids*, *citric*, *malic and succinic*.

Succinic acid is not very common and so we need consider only citric and malic acids. The presence of these particular acids with maximum buffer indexes above pH 5·0, will tend to narrow the zone of minimal buffer capacity i. e. the valley in the curve of the buffer complex (see figs. 19, 27). Malic acid will tend to raise this minimal zone between pH 5·5 and pH 6·0 (cp. ÚLEHLA 5·6) while citric acid will tend to raise it still higher, nearer to pH 6·0 (cp. YOUDEN and DENNY 6·2, and INGOLD 5·8 p. 281). The relative buffer capacities and relative concentrations of the systems above and below the minimal β zone will determine its exact position on the pH scale. The citric acid system in moderate concentration might easily dominate a moderate phosphate-bicarbonate complex, obliterating the hollow in the β curve altogether with unit-pH grouping as in the potato (fig. 20).

Thus we find that, although we know very little of the actual buffer systems present in plants, there are few buffer systems which could possibly produce anything but a buffer complex with a minimal buffer capacity somewhere between pH 5·0 and pH 6·2; one of these being a citrate plus phosphate system (see & cp. figs. 19 and 20).

Again the basis for attributing an important rôle to carbon dioxide in the control of the internal pH is strengthened. Where the β curve of the expressed juice slopes downwards all the way from pH 4 to pH 7·5, there would appear to be nothing available to keep the natural pH of the cell fluids below pH 8·0, except the carbon dioxide content which is present in the living cell but largely absent from the expressed juice.

Experimental. — The change in reaction produced in sunflower, bean and potato saps in equilibrium with various concentrations of carbon dioxide are tabulated on pp. 259 and 289. The necessity for determining these changes in a closed system should be quite clear since volatilisation of the CO_2 on exposure to air may lead to a change from pH 4·8 to pH 5·6 (p. 218). As a matter of fact, using a SPARKLET siphon and carbon dioxide at more than one atmosphere pressure, a reaction below pH 3·4 can be obtained by means of this acid alone. The above-mentioned data were obtained by Miss MARTIN, for the sunflower and bean, using tintometer bottles and the tubes from a GANONG's photosynthometer; but INGOLD for the potato improved the manipulation by adapting the apparatus for capillator determinations. The details should be quite clear from figure 25 and legend.

The reaction changes found are given in Chapters XII—XIV, and here in fig. 26. It will be obvious that they are large enough to be of significance in the life of the cell, which is carried on with an intercellular atmosphere frequently much richer in carbon dioxide than the free air outside the tissue. MAGNESS (1920) found the intercellular carbon dioxide content to be 6·7—21·4 % in apples, 12·2—28·6 % in carrots and 19·6—34·4 % in potatoes, with temperature as one of the dominant factors in CO_2 production.

The curves in fig. 26 are practically titration curves of the sap with carbonic acid. The sunflower curve **AA** shows the result of very weak phosphate buffering (giving a rapid fall of pH with 5—10 % carbon dioxide) combined with little organic acid (cp. fig. 20C); the bean curve **BB** shows the effects of phosphate buffering with malate effects active above pH 4·8 but reinforced by the steep oxalate buffer increase below that reaction so that the

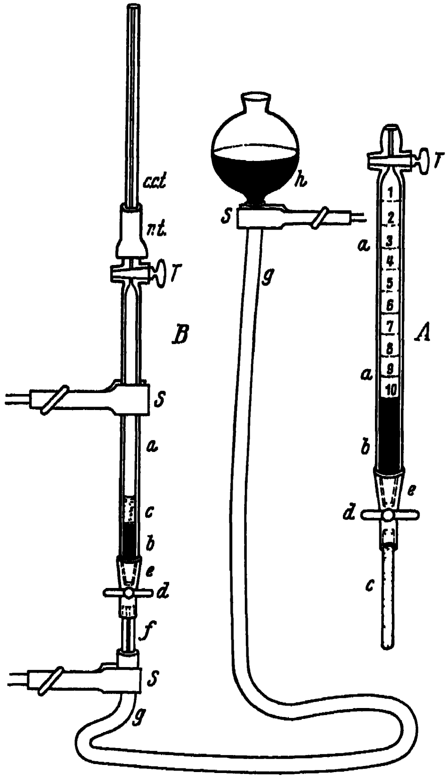

Fig. 25. Apparatus for studying Carbon Dioxide Effects on the pH of plant juices. **A** — Ganong's photosynthometer tube, with **a** a known atmosphere of carbon dioxide and air drawn in above mercury **b**: a 2cc. tube **c** full of sap plus an equal volume of capillator indicator is attached with the screw-clip **d** closed. The opening of **d** allows the sap to pass through the rubber tube **e** while the mercury flows below. The clip **d** is then closed and the sap shaken with the carbon dioxide for several minutes. **B** — a capillator capillary tube **c.c.t.** is attached by means of rubber tubing **r.t.** to the upper end and a mercury reservoir **h** with pressure tubing **g** is connected by means of a thick-walled capillary tube **f** to the bottom below the clip **d**. The mercury reservoir is placed as figured so that the sap **c** is brought *slowly* up to the tap **T**, now open, and into the capillary tube **c.c.t.**, where the tint can be compared with those of the standard capillator cards. **ss**-screw-clamp supports.

curve is quite flat (cp. fig. 19); while the potato curve CC shows the result of phosphate plus citrate with very little oxalate present (cp. fig. 21).

Bolas (1926) gives a convenient apparatus for the measurement and control of carbon dioxide content of a closed system in which natural conditions as regards that part of the environment might, with advantage, be used in many permeability studies. The relative merits of electrometric and colorimetric measurements are discussed by Cullen and Hastings (1922) but it should be quite clear by now that colorimetric methods are best in relation to such large concentrations of carbon dioxide. Bolas used brom-cresol purple and found a mean error of 1·85 in 10,000.

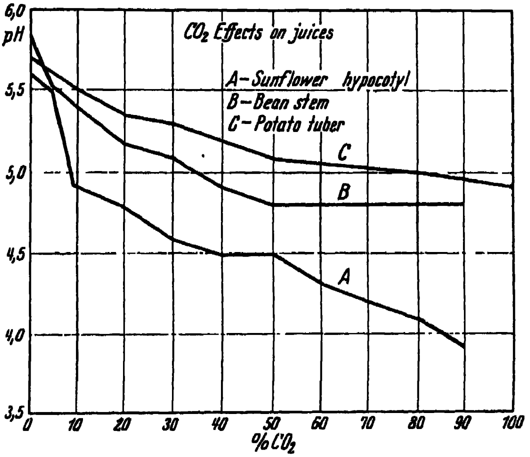

Fig. 26.

Carbon dioxide effects have been studied in various connections e. g. Lillie (1909) on stimulation; Kidd (1919) who found potato sprouting inhibited by an external concentration of 20 % CO_2; Crozier (1919) found a marked internal tension of CO_2 in *Valonia* sap, giving marked changes in [H˙];; Irwin (1919) and Jacobs (1920) on external acidity and CO_2; Gustafson (1920), McClendon (1920) and Smith (1924) on external CO_2 and respiration; Mottram (1928) who found a partial pressure 160 m. (about 20 %) of carbon dioxide in cancerous tissues;

Thomas (1925) who found that carbon dioxide, even in the presence of abundant oxygen, may cause the respiration of apple cells to be changed to a zymasic type; **Noyes** and others (1918, 1920) who found the shape of the root systems of various plants altered; and finally **Jacobs** (1922) who found important effects upon the physical condition of the protoplasm.

There are many other aspects of this — see **Smith** (1923) on petal colours; **Sakamura** (1922) and **Scarth** (1924) on the toxic action of distilled water, traced by the latter to the pH effects of dissolved and unbuffered carbon dioxide. **Olsen** (1923), **Bennet** (1927) and **Úlehla** (1928) are amongst the many who have found similar effects with unbuffered or weakly buffered experimental fluids. Enough, however, has been given here to prove the relative importance of these carbon dioxide effects under natural conditions of plant cell life, see also Chapter XIV 'Wound Carbon Dioxide', 'Effects of Sectioning', etc.

(c) *Acid-producing Metabolism.* Fungi, yeast and bacteria, by acting upon various proteins, amino-acids and carbohydrates produce a variety of organic acids such as formic, acetic, propionic, butyric, caproic, isovalerianic, malic, succinic, phenyl — and indole — acids etc., (cp. **Cameron** 1928 pp. 168 sqq. and **Czapek** 1925 III p. 109). Citric acid and malic acid are common in fruits of *Rosaceae* and *Ribesiaceae* as well as in citrus and *Vaccinium* fruits (**Czapek** III pp. 107-8).

The various changes which these organic acids may undergo in the presence of nascent oxygen or nascent hydrogen can be appreciated better after a perusal of some treatise on electro-organic chemistry such as **Brockman** (1926).

It is, therefore, well known that intra-molecular changes and incomplete oxidations of carbohydrates frequently result in the formation of organic acids. In the normal plant-metabolism oxidation is complete to the stage of water and carbon dioxide. This carbon dioxide can pass through the cytoplasm and to a great extent out of the plant tissue. It is quite otherwise with the organic acids produced by what we may term abnormal metabolism. Some of the weaker of these may pass easily through the cytoplasm, e. g. benzoic, salicylic, propionic and acetic acids, but the cytoplasm is almost impermeable to the stronger commoner types such as oxalic, citric, malic and tartaric acids (cp. **Czapek** III p. 110 *con* and **Wertheimer** *pro*), at any rate so long as they remain free acids and do not become more or less neutral undissociated salts of these acids. If, therefore, these acids be produced within the cell vacuoles they will be largely retained there as acids.

The consequences of this retention of free acid in the vacuole appears to have little or no effect as such upon the form of the plant or consistency, succulent or dry, of the foliage, see Chapters X—XI. WAKSMAN, however, states (1928, p. 2281) that "oxidizing processes have not been definitely proven to be enzymatic in nature; for example, the laccase of *Medicago sativa*, instead of being an oxidase, is sometimes referred to as a mixture of salts of organic acids and alkali earths; glycollic ($CH_2OH \cdot COOH$) and glyoxalic ($CHO \cdot COOH$) acids predominate among the former," also (ibid p. 232) "substances like oxygenase and peroxidase, only recently considered as oxidizing enzymes, begin to lose the attributes of enzymes."

The organic acids and their salts in the cell sap would appear as possibly important constituents of plant cells; quite apart from their undoubted roles — (1) as very effective buffer substances capable of keeping the reaction of the sap within the limits of the range which the adjacent cytoplasm can endure without serious injury, and (2) as acids producing the various degrees of [H˙] which determine the relative activity of enzymes in an enzymatic mixture.

For example, we write of these free acids being confined to the vacuoles. Suppose that free organic acids be formed in the cytoplasm, competition between these acids and the other base-containing substances in the cytoplasm will at once develop, whether the bases are adsorbed by lipoids like the calcium which reacts to give HEILBRUNN's ovothrombin (1928, Chapter XIII—XIV) or whether they are bound chemically, a certain proportion of base will be transferred to the freshly formed organic acid. But in spite of this degree of neutralisation the reaction will be changed locally, a flow of calcium or other base to give an internal surface-precipitation membrane may be postulated, and the acid vacuole is formed; once formed it will maintain itself by the difference in [H˙] with the resulting demand for base and attraction of calcium amongst other bases. Within limits, a fresh secretion of acid by the cytoplasm or an increase of acid within the sap system, would result in an enlargement of the vacuole and the formation of a larger precipitation membrane around it. The acid, in fact, would tend to remove the calcium from the membrane until an equilibrium was established, with fresh formation of the membrane from the cytoplasmic phase of the system.

(d) *Oxalate Crystals.* Oxalic acid is one of the commonest organic acids produced by the above-mentioned abnormal metabolism. The pK values for this acid are 1st 1·42 and 2nd 4·39. Free oxalic acid is stronger than sulphurous acid and much stronger than any other common organic acid with the exception of maleic acid (pK 1·93; not known in plants, Czapek III p. 87). The control of [H˙] due to oxalic acid becomes, therefore, an important problem for the plant cell where the cytoplasm is usually injured by reactions which fall below pH 2·5 as a minimum. Injury is common at much smaller hydrion concentrations. The control by salt-formation and buffering with an acid-salt plus normal-salt system around the second step (pK 4·39) would be effective but an acid plus acid-salt buffer system would be of very little use around pH 1·42.

The calcium flow for surface precipitation has been mentioned above, and while the oxalates of sodium or potassium are very soluble, calcium oxalate is almost insoluble at a [H˙] above pH 1·5. Even with sodium or potassium present, therefore, the oxalate would tend to become entirely calcium oxalate and to crystallise out. Below pH 4·0 the bulk of the salt would be the acid oxalate $Ca(HC_2O_4)_2$ giving monoclinic crystals (see p. 102) which would be large on account of the concentration and slow crystallisation; near pH 5·0 the same result might be expected together with the appearance of a certain proportion of tetragonal crystals of CaC_2O_4 small on account of the lesser concentration of CaC_2O_4. Beyond pH 5·0 the proportion of acid salt would decrease continually, until at pH 6·0 there would be few or no monoclinic crystals, and the normal salt with larger tetragonal crystals would be predominate. The drusy crystals mentioned by Pfeiffer (see p. 101) may be a hydrated form of the normal salt, or their formation may depend upon the relative solubility or speed of formation in the various media which he used. There is some evidence also that the monoclinic salts have two, while the tetragonal salts have six molecules of water of crystallisation (Czapek III, p. 68).

In any case, we have two interesting results —

1. The formation of oxalic acid is accompanied by vacuole formation and the crystallisation of calcium oxalate. The cytoplasm is thus protected from an injurious [H˙] of pH 1·42 or lower.
2. By observing carefully the crystal form of calcium oxalate we can arrive at an approximation to the pH of the sap of the cells in which they were formed.

(e) *Colloids in the Cell Sap.* There is no evidence of the occurrence of colloids in the vacuolar sap of ordinary plant cells, but there is no doubt that special cells, such as the guard-cells (SCARTH p. 92), hypodermal and bundle-sheath cells in *Bryophyllum* (LYNN p. 127), hairs of many plants, etc., occur which contain colloids in their vacuoles. These in many cases are hydrophilic colloids such as gums, mucilages, pentosans, gelatinous and pectin substances, proteins and phosphatides. Normally these colloids are confined to the cytoplasm and the cell wall, but in these special cells they invade the vacuole.

The effects of [H˙] changes on the non-amphoteric colloids are still obscure. MAC DOUGAL's mass of data (1920) yields little of precise value, on account of the mixture of amphoteric and other colloids used and the almost complete absence of precise pH values for the mixtures of acids and salts used. Proteins would appear to be rare in vacuolar sap (see below) and phosphatides, although they are amphoteric (see CAMERON 1928, PRYDE 1928, also MAC DOUGAL and MORAVEK 1927), are so recent that no certain data are available as to their occurrence in sap. The increase of the hydrophilic forces of proteins and other amphoteric colloids with increase or decrease of [H˙] around a particular zone is well known. The other colloids are hydrophilic by virtue of their surface charges and it would seem theoretically probable that changes in the density of the charge would result in changes in the hydrophilic properties. Accurate work is necessary, but the phenomena of colloids in the sap are not of general significance, although they may be important in special cases, as in guard cells of stomata and in some succulents.

(f) *Proteins in Cell Sap.* Considering the very small quantities of protein found in expressed juices by various workers (e. g. HURD-KARRER, YOUDEN and DENNY, MARTIN and INGOLD) and that these juices are crushed cells with cytoplasmic admixture, it would seem that proteins in the vacuolar sap can be regarded as almost negligible.

(g) *Equilibrium Points and Buffering.* As has been already pointed out (p. 322 and p. 331), in the absence of citrate buffering there is normally, in a buffer complex of organic acids and phosphates, a minimal β zone which varies in position but is usually in the range pH 5·0 to pH 6·2. With oxalate buffering combined with a strong phosphate concentration the minimal β zone may go lower.

In the form of more or less neutral salts these buffering substances may diffuse outwards. For example, in the work of HIND (1926) and STILES and JORGENSEN (1915) an increase in the external electrolytes (increased conductance) was found to be associated with the decrease in external $[H^{\cdot}]$ and a re-interpretation of the data upon the basis of an outward diffusion of buffering substances (cp. STILES 1928, on exosmosis), instead of an absorption of hydrogen-ions, might prove to be a valuable contribution to this aspect of the subject. As another example, the substances indicated, but not identified, by YOUDEN and DENNY are such as would normally occur in the sap rather than in the cytoplasm.

Thus we arrive at the conclusion that the buffer systems of the vacuolar sap should be known both qualitatively and quantitatively before any attempt is made to explain $[H^{\cdot}]$ phenomena, external or internal, by appeal to the unknown properties of an unknown cytoplasmic mixture. If the known action of the vacuolar sap explains the observed phenomenon we can rest satisfied; if not we may then suggest cytoplasmic effects.

(h) *Membrane Buffering; Sap and Cytoplasm.* We have seen above (p. 323) that cytoplasm exerts a buffer action by preventing the passage of stronger acids or bases. This may result in acid vacuoles or even in some vacuoles being acid with others relatively alkaline (cp. METCHNIKOFF), while the cytoplasm remains somewhere between pH 5·0 and pH 6·2 (cp. HOAGLAND and DAVIS 1923). Given the large protein and phosphatide content with the consequential large buffer index of the cytoplasm there is nothing unexpected in this arrangement.

The acids of the vacuole cannot *as acids* pass into a strongly buffered medium of higher pH. The pH of the cytoplasm is held unaltered by the buffer capacity of the cytoplasmic mixture so that an acid system entering this medium has its pH raised, with a consequent change in the acid-salt ratio towards that which occurs at the pH of the cytoplasm. The acid system becomes mainly slightly acid salt plus neutral salt, and in this condition it may or may not pass through the cytoplasm. The balance of the evidence is in favour of a certain degree of permeability of the cytoplasm for (neutral ?) citrates, malates and tartrates, while oxalates as we have seen are crystallised in the vacuole in the form of calcium salts. The relative solubilities of potassium and calcium salts in the vacuolar sap are not sufficiently different to lead to the exclusive formation of calcium salts of the other acids.

The cytoplasm and sap are, therefore, to be regarded as quite separate systems from the pH point of view, with a possibility of the constituents of the sap passing outwards through the cytoplasm, but with little likelihood of cytoplasmic invasion of the sap system in the normal cell and practically no possibility of such a mixing in the case of cells with strongly acid vacuoles.

CHAPTER XVIII

CELL WALL AND pH

1. The real pH of Cell Walls. — (a) errors of methods; (b) pH values recorded; (c) pH values in relation to errors; (d) can the pH be determined? (e) the evidence.
2. pH and Cell Wall. — (a) cellulose; (b) lignin; (c) suberin; (d) cutin; (e) equilibrium points; (f) membrane buffering; wall, cytoplasm and sap.

1. THE REAL pH OF CELL WALLS

Whereas the conditions in the sap appear to be similar to those of free aqueous solutions and those in the cytoplasm are dominated by protein and phosphatide colloids, the plant cell wall must be regarded as usually a cellulosic colloidal gel with aqueous solution in the disperse phase, with variations caused by admixture of pectin substances and other types of substances such as lignin, suberin and cutin, which further may form more or less pure layers of non-cellulosic nature. The determination of the pH of such material presents considerable difficulties.

(a) *Errors of Methods.* Some of the methods applicable to the vacuolar sap can be applied to the *aqueous fluid of the walls* and the same errors will be possible. Using microscopic examination of sections or pieces immersed in indicator fluids there will be several other errors of special importance in connection with the wall fluids. Expressed sap results can be neglected entirely and hydrogen-electrode methods would appear rather impossible.

Using the R.I.M., the main difficulty is that the wall fluids are free to mix with the indicator fluid. Unless the wall fluids are moderately acid in reaction and moderately buffered, they will show the general colour of the immersion fluid even after being rinsed in neutral water. This is the neutral colour in all cases and so there will be always indeterminate results.

The *solid phase of the wall* is naturally insoluble in water and will not show the normal dissociation phenomena of aqueous solutions. The R.I.M. letter symbols are, therefore, used throughout the discussion of solid phase colour records. Indicator dyes may not be taken up by the wall at all, or they may be absorbed or adsorbed to a varying degree. The actual tint of the indicator in this case will depend upon various factors, such as time of immersion, time of washing, physical condition of the wall and its chemical composition. Tint-comparison methods are, therefore, to be avoided completely.

Colour indications, as used in the R.I.M., avoid the errors of tint-comparison methods, and definite colours are obtained for some types of cell-wall, solid phase as distinct from wall fluids.

The lipoid error, which may be important in some cell walls, has been discussed in detail on page 30, and is further considered below on page 344.

(b) *pH Values Recorded.* Atkins gives some data for cell walls which are, so far as definite colours are concerned, in agreement with those found by the R.I.M. (see p. 98). Throughout the R.I.M. survey it was found that cellulosic walls never gave distinctive colour differences, except in the potato and when the cell-sap was of the acid type as in the various acid families. The colour-changes in the walls of *Pelargonium* and some other generally acid stem tissues corresponded closely with those given by the sap, but the colouration was not so deep; and a clear colour difference was found under certain conditions for the potato tuber and in *Taxus*. This may indicate that the wall fluids of cellulose walls are more or less of the same pH as the cell sap. Where the cell-sap is yellow with diethyl red and yellow with brom-cresol purple (pH 5·9) the faint yellow is very difficult to distinguish from the natural colour of the wall and still more difficult to distinguish from the neutral tint of the indicator fluids.

The other wall-colour changes obtained may all apply to the solid phase of the wall and, with a few exceptions, indicate relatively acid walls. These relatively alkaline exceptions are —

1. The alkaline epidermal hairs of the sunflower (p. 192). These are hairs with a multicellular base; the long apical cell gives a deep blue with B C.P. and B.T.B. and shows the alkaline colouration for P R., N.R., C.R. and thymol blue, but is colourless with phenol phthalein, thus indicating an apparent

pH between 9 and 10. This is the highest value recorded throughout our survey and appears to be quite exceptional.

2. The callus-blocked sieve plates of the phloem in the sunflower, which are blue with B.C.P. but show no trace of blue with B.T.B., indicated range C (pH 6·2 *ca*). The contents of the same cells varied from the range pH 5·2—4·8 up to pH 5·9 *ca*. This *wall* structure appears, therefore, of a similar reaction to the sap of some algae and fungi (see p. 93). There is no evidence, however, that the sieve-tube *contents* are normally alkaline. The *contents* are, in fact, frequently as acid as pH 5·2—4·8 and have not been observed to be higher then pH 6·2 *ca*.
3. The walls of the sieve-tubes in *Equisetum* (see p. 96) which gave the same reaction C as sunflower callus-plates, with contents also at pH 6·2 *ca*.

The *acid colour-changes* were obtained mainly in lignified, suberised or cutinised walls, the acidity indicated was usually in the range **h** (pH 4·4—4·0) passing sometimes to **k** (pH $<$ 3·4), beyond which point wall reactions have not been investigated. It should be observed that the lowering of the apparent pH with progressive lignification can be studied in a series of sections from the tip of the stem downwards. Suberised walls also gave colours indicating range **k** (p. 194); cuticle varied from **e** (pH 5·2—4·8) down to **i** (pH 4·0) or even to the **k** range.

Acid colour changes, giving faint pink with DER and MR, and alkaline colours with all the lower indicators (range **e**) were also found frequently in collenchymatous walls, and occasionally in sieve-tube walls and some apparently cellulosic walls of parenchyma.

(c) *pH Values in relation to Errors. Liquid Phase.* — A consideration of these data shows that the pH of the fluid phase of the wall can be determined with as reasonable a degree of accuracy as can that of the vacuolar sap, provided that the fluid is acid to DER or MR or both; and that the pH of a wall fluid between 5·6 and 6·2 is very difficult to observe.

Less Acid Walls. — When the colour changes of the solid phase of the wall are considered we come at once to the most obscure problem of tissue reactions. The structures giving relatively or actually alkaline reactions do not appear to be soluble in water, but it is possible that we are dealing here (a) with relatively alkaline wall fluids or (b) with bases adsorbed by the wall but still capable of exerting a strong buffer action so that the carbonic acid or other weak acid of the fluid phase does not produce acidification. Phosphates are not impossible (cp. CZAPEK I p. 681); as calcium phosphate they would be relatively insoluble in the aqueous phase. Pyrophosphates and organic phosphates appear a possibility, especially after the recent work of LOHMANN (1928). Other possible sources of the relatively alkaline solid phase are the phosphatides, of which lecithin and kephalin do not appear very probable, while sphingomyelin is not yet known in plants (PRYDE p. 204).

The ether soluble, nitrogen-free calcium phosphatide isolated by CHIBNALL and CHANNON (1927) appears to be a distinct possibility since it occurs in plants (cabbage and *Ricinus* leaves) and shows an excess of unsaturated over saturated fatty acids. The fatty acids of this phosphatide are linolic, linolenic, stearic and palmitic. As a calcium salt this phosphatide might give the colour reactions observed. Further work on this problem is suggested as of interest.

More Acid Walls. — While it is possible that the less acid walls are wet in the solid phase, the lignified, suberised, or cutinised wall substances are generally regarded as immiscible with water. Normal ionisation phenomena are to be considered as absent from the more acid solid phase of the wall. The indicator dyes penetrate these acid walls and show colour changes, 'acid' colours with some indicators and 'alkaline' with others. Apparently '*something*' is being measured which is greater in relation to the higher indicators (BCP, DER, MR) and lesser in relation to the lower indicators. There appears to be a progressive increase of this '*something*' with maturing of the cell-walls concerned, so that '*it*' gradually becomes greater even in relation to the lower indicators (BAN, BCG and BPB) and then gives 'acid' colours with these indicator dyes.

The absence of water from these coloured solids possibly raises the problem of the dielectric constant and its influence upon ionisation phenomena. The presence of fatty acids and or salts or esters of fatty acids means that this constant would be very low. With water at 81·7, acetone at 20·7, toluene 2·31, benzene 2·26, lignin and suberin would probably be lower still. The degree of ionisation of the indicator dyes in the walls would appear to be negligible. The '*something*' which is measured is clearly not ionic concentration, whether ionisable hydrogen does or does not enter into these colour changes in acid cell walls.

It is here suggested as a possibility that the colour changes with acid cell walls are due to reactions between indicators and the fatty acids or anhydrides of the walls. Thus, if the acids of the wall are stronger, (not in their ionisation but in their avidity for the alkali base of the indicator-salt), than the acid of the indicator-dye, the base of the indicator will pass to the wall-acid, even in a relatively non-ionised mixture, leaving the indicator-acid in the free condition. Whether this indicator acid shows the same colour under these conditions as it does in aqueous solution is one of the chief problems still to be solved, and work on these lines is proceeding in this Department.

Pelargonic acid, $CH_3(CH_2)_7COOH$, may be mentioned as an intermediate member of the acetic-stearic series. This acid is an oily liquid, soluble in water only to the extent of about ·0025 per cent w/v or, ·00002 molar. The aqueous solution gives acid colours down to BCG and alkaline colours with BPB and thymol blue (acid range), indicating a pH 4·0 *ca*. An excess of the acid shaken with aqueous indicator solutions takes up the indicators and shows distinctly the acid colours with neutral red, PR, BTB, BCP, BAN, BCG, B.P.B., and thymol blue (acid range), corresponding to pH 1·4. The colour given by MR and DER is neutral with pure acid and aqueous indicator solution, but the acid mixes freely with alcoholic indicator solutions giving the deep red colour. When this alcoholic mixture is diluted with three or four volumes of water the acid separates out carrying a deep red colour into the oily layer. The action of mixed or impure solvents may be effective, and the wall substances may exert a similar action.

The acid type of wall substance concerning which the most definite information is available is suberin. Lignin still suffers from the hadromal controversy[1]), although the cerebroside and phosphatide affinities of lignoceric acid may lead to a solution of this problem (cp. CZAPEK III, p. 790). Cutin is similarly obscure. Concerning suberin, SCURTI and TOMMASI (1913, 1916 see

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[1]) For a convenient summary of the lignone complex, see DORÉE and BARTON-WRIGHT (1929).

(Czapek I, p. 698, III, p. 790) have shown that (1) the so-called phellonic acid is *α*-hydroxy-behenic acid $C_{22}H_{44}O_3$ or $CH_3(CH_2)_{19}$-$CHOH \, . \, COOH$ a hydroxylated derivative of one of the acetic series $C_n H_{2n} O_2$; (2) the so-called suberinic acid is ricinoleic acid, $C_{18}H_{34}O_5$ or $CH_3(CH_2)_5CHOH \; CH_2 \cdot CH = CH \cdot (CH_2)_7 \cdot COOH$, a hydroxylated derivative of one of the oleic series $C_n H_{2n-2}O_2$, probably oleic acid itself which written to show the similar structure is $CH_3(CH_2)_5 \cdot CH_2 \cdot CH_2 \cdot CH = CH \cdot (CH_2)_7 \cdot COOH$. The third fatty acid of suberin so-called phloionic acid, is according to Scurti and Tommasi a tri-carboxylic acid with 25 carbon atoms, derived from the usual fatty acids by oxidation.

While these acids are present partly as salts in the walls, the free condition in part seems indicated and the maturing changes would then be progressive dehydration with formation of possibly more complex and more acidic anhydrides. Herklots (1924) found the fatty acids in potato-wounds more mobile at low pH and more easily oxidised at high pH values, which latter promote the fixation of the fatty acids in the walls. The hydroxylation and the double bond are both factors which give a greater avidity for base to these fatty acids. The use of Nile Blue, which changes at a high pH, as a test for free fatty acids and *neutral* fat would appear significant in this connection. We are led, therefore, to the tentative conclusion that, using the R.I.M. on acid cell walls, we measure, not the pH, but the strength of the various fatty acids present. Further work is being done to test this hypothesis but it must be remembered that here we are working in the region concerning which Clark (1928, p. 7) writes, "Even beyond the measurable lie cases for which the presumption of ionisable hydrogen is often useful."

(d) *Can the pH be determined?* As with cytoplasm and vacuolar sap the answer to this question cannot be an unqualified Yes or No.

The pH of the liquid phase can be determined by means of the R.I.M., if the wall fluids are acid (below pH 5·2), but not if they are between pH 5·2 and pH 6·2, the points at which methyl red goes distinctly red and brom-cresol purple goes distinctly bluish respectively. Beyond pH 6·2 the problem of whether the colours are due to the liquid or the solid phase or both remains unsolved. The examples are very few, and this forms one of the special investigations which were looked for during our general survey.

The pH of the solid phase cannot, in the ordinary meaning of the symbol, be determined at all in the absence of normal ionisation; but by means of the R.I.M. we can measure something which may be the base avidity of the acids concerned, and after all hydrion concentration is only one way of measuring the strength of acids.

(c) *The Evidence.* In connection with the reaction of walls to indicator dyes the main evidence has been obtained during the R.I.M. survey and is detailed in Chapters X—XIV. The evidence, therefore, is entirely concerned with the reaction of the wall fluids and solid substances with indicator dyes, and its significance varies with the phase, fluid or solid, and with the apparent reaction relatively alkaline or relatively acid. This has been discussed above.

2. pH AND THE CELL WALL

As with cytoplasm and sap we can distinguish clearly between the actual or apparent pH of the cell wall and the influence which the cell wall has upon the phenomena of hydrion concentration in the cell or tissue.

(a) *Cellulose and allied materials.* According to Odén (1916) the ordinary cell wall includes what he classes as pectin-substances. A number of plant mucilages and gums were included in this group on account of their known acid nature and their small 'real' solubility. The pectins as defined were considered to be important not only as binding materials but also as pH regulators. Odén discussed the effects of acids and alkalies mainly from the point of view of decomposition of calcium pectate and the swelling of the pectin thus liberated.

According to Priestley (1924) "fats, fatty acids or soaps can be detected in the walls of plant cells from a very early stage and appear to be present as early as the cellulose and pectic substances regarded as typical of the plant wall." The same author emphasises the physiological importance of the fats and phosphatides found by Hansteen-Cranner in "the membrane of normal parenchymatous tissues."

A summary statement of the effect of acids in bursting cells of various kinds is given by Lloyd and Ulehla (1926). The material used includes pollen grains and pollen tubes of *Phaseolus odoratus*, hyphae of the fungus *Basidiobolus ranarum* and apical regions of the algae, *Trentepohlia*, *Cladophora*, *Vaucheria*, *Bornetia* etc. The high speed of action and the fact that the acid bursts the cells before it penetrates and coagulates the cytoplasm, in *Bornetia* changing a natural indicator in the cell, led ÚLEHLA and MORAVEK (1922) to ascribe the main rôle in the explosion to the cell wall. They considered that adsorption of the hydrogen-ion by the wall changed suddenly the degree or sign of the electric charge. The cytoplasm of *Basiobolus* is almost or quite impermeable to strong acids, including ·00005 N HCl which gives bursting in 30—60 seconds from first contact.

LLOYD, for pollen grains and tubes, concluded that the acid penetrated both wall and cytoplasm rapidly giving first a swelling of the cytoplasm (leading to bursting) followed by coagulation. LLOYD and ÚLEHLA, in collaboration, came to the conclusion that the sudden bursting in acid "is directly connected with sudden changes in the biocolloids whether they compose the cell wall or the protoplasm." Methyl red was used as an internal indicator for pollen grains and changed from yellow to red within ten seconds or less of the first contact with 1/800 N HCl in 1·5 M sucrose, but coagulation was found on immediate crushing of the grains under the cover glass.

Later work, detailed in the abovementioned joint paper, led these authors to the conclusion that in the stipe of the marine alga *Postelsia palmaeformis* "some irreversible change similar to death happens not only in the protoplast, but also in the cell wall." "Swelling curves obtained with dead material indicate a behaviour approximately between that of agar and of gelatine gels with strong evidence of an isoelectric point at ca. pH 5·6." This isoelectric point of dead material is probably not unconnected with the agar-like algal material used, but the "death" of the wall as well as of the cytoplasm introduces the conception of a "living" cell wall in which any or all of the pH phenomena of cytoplasm as described in Chapter XVI may apply. There might be a similar dominance of ampholytic colloids, as agar-like proteins or pectins or phosphatides with a similar strong buffering even in the region of the normal pH range, because of the large concentration of buffering substance; but strong buffering does not agree with the rapid penetration of the wall by strong acids noted by both these authors. Strong buffer action in any zone or film means relative insensitivity and relatively low permeability to all strong acids or alkalies, at least so long as the zone or film retains its original buffer capacity.

We are, therefore, led to the conclusion that the ampholytic colloid content of the wall must be small enough to give a low buffer capacity, while it is large enough to determine the electro-surface phenomena such as the sign and density of the charge, an alteration of which gives rise to the 'death' phenomena observed.

With the electrically neutral general cellulose frame-work we must clearly be prepared to have other substances which may have a marked effect upon the reactions and permeability of the wall to fluids of varying hydrion concentration. Further, the same abnormal metabolism which results in vacuolar sap below pH 5·2 may result also in the impregnation of the cellulosic wall with acid 'pectins', so that the acid reaction observed in *Pelargonium* stem and other generally acid tissues may not be due to the fluid phase of the wall but to these acidic solid materials. The same phenomena may be responsible for the slightly lower acidity (e) of collenchyma, e.g. in *Lamium* p. 143 and *Senecio* p. 146.

(b) *Lignified Walls*. ROHDE did not distinguish between the various parts of the cell, but ATKINS (p. 98) records sclerenchyma as pH 5·2—5·4, wood walls at pH 5·4—5·6 for *Salvia* and *Cochlearia*. During the R. I. M. survey the following points were noted —

1. The unlignified vessel walls of *Hedera* hypocotyl were in the range **b**.
2. The lignified walls of vessels and tracheids in young flowering stems were almost without exception in the range **h** or lower in **k**.
3. That the lignified walls become more acid on maturing is shown by many records, (a) where the acidity increases from the upper part of the vegetative stem (**h** or **g**) to the lower (**hk** or **i**) as in several twigs of *Aucuba* (p. 146) *Ligustrum* (p. 148). *Rhododendron* (p. 151) and notably in *Veronica* (p. 153) and *Viburnum* (p. 156) where this phenomenon was very general.
4. In *Helianthus* (pp. 186—187) the vascular strands are acid (pH 4·4—4·0) before lignification begins, but the xylem walls were specially noted as in the range **e** while unlignified, changing to **h** at the expansion of the cotyledons (Table II, p. 186). The lower range **k** for xylem walls appears only at the mature

stage in the stem below ground, and for xylem fibres in the older part of the root at a similar age.

5. In *Vicia faba* (pp. 230—231) the procambial strands were less acid in the seed (pH 5·2—4·8), but changed to **h** (pH 4·4—4·0) in the root immediately on germination and in the stem before the radicle was 4 cms. long. Again the xylem fibres and also the pericyclic fibres (p. 235) of the mature plant were in some cases found to be in range **k** near the base of the stem (p. 233). Otherwise both sunflower and bean have the lignified walls of the xylem in the **h** range.
6. The pericyclic zone is frequently lignified in parts, giving **h** or **i** or **k** for the lignified walls and pH 5·9 to pH 5·2—4·8 or even pH 4·4—4·0 for the cell contents in the unlignified parts of this region, see numerous examples in Table VI Chapter X, and Tables I, VII and VIII, Chapter XI. The increase of acidity in maturing was noted in *Viburnum* (p. 156).
7. The pericycle in *Helianthus* is of special interest. In the upper parts of the stem, fibres were not observed by MARTIN (p. 186) and the reaction of the rest of the stem was found to vary from pH 5·9—5·6 to pH 5·2—4·8. Fibres appeared in the lower parts of the stem and the upper part of the root, at the third or fourth stage as **h** passing to **hk** or **k** in the mature plant in all these parts of the plant.

INGOLD and SMALL (1928, see p. 57) record the presence of a group of cells in the sunflower, which occur in the cortex opposite the groups of pericycle fibres. These cells, some of which may be actually in the endodermis, show very decidedly red with methyl red (pH 5·2). They have been investigated in detail by C. T. INGOLD (unpublished work) and have been found to show very small intercellular spaces filled with an oily fluid which readily takes up the (neutral ?) form of the indicator. This oily fluid in the intercellular spaces may or may not be an excretion from the acid cells, and it could apparently travel *via* the endodermal walls to the lignifying walls of the pericyclic fibres just within the group of special acid cells. This forms another problem for further investigation.

These items for lignified cell walls are in general agreement with the suggestions made above (p. 159). An actual influence of pH, as such, upon lignified walls or upon the process of lignification would appear to be very slight or altogether absent, although acid cell contents would appear to precede lignification. Possibly this content-acidity like that of succulents, is another example of the same cause (abnormal metabolism) giving two mutually independent results. Pentosan formation and organic acids in succulents may be paralleled by organic acid formation and lignification in xylem, pericycle and sclerenchyma.

(c) *Suberised Walls.* The R. I. M. survey was conducted upon material from which cork was as a rule absent. Phellogen was investigated in the sunflower by MISS MARTIN (p. 196). She found that the cell contents of the actual cork cambium were at pH 4·4—4·0, while those of the cells out off towards the outside were at pH 5·2—4·8. The dead shrivelled cells to the outside of these gave a lower reaction. Later stages were not observed. More mature periderm in *Sambucus* stem gave pH 5·2—4·8 for phellogen contents with the range **k** in the suberised walls of the cells on the outer side of the phellogen.

Suberin occurs also in the walls of the endodermis. The wall reactions of the endodermis are not recorded frequently; but **h** and **g** occur for *Cerastium* (p. 137). In *Helianthus* the endodermal contents had a pH similar to that of the cortex in the upper parts of the stem, but below ground, in stem and root, the records indicate acidification and possible extensive suberisation of the walls to the ranges **h** or **k**.

Suberin is a waterproof material, the importance of which in the life of the plant has been emphasised by PRIESTLEY and his collaborators in a series of papers (see The New Phytologist 1921—1924, also Proc. Roy. Soc. B. 100, p. 119). The maturing changes noted for lignified walls are here not so obvious, and it is possible that the actual fatty acids found (see p. 345) give the dye-reactions and its other properties to suberin, while any anhydride formation would appear to be more rapid in this case than with 'lignin'.

(d) *Cutinised Walls.* The acid colour reactions of cuticle with methyl red were noted from the beginning of the R. I. M. survey, but the degree of acidity was found to vary; thus it is **e** for stems in *Reseda, Phyllocactus, Senecio, Lapsana*; **h** for stems in *Cheiranthus, Ligustrum*; **g** for *Haworthia* leaf, *Euphorbia* stem, *Aucuba* stem; **i** for *Echinocactus*; **k** for *Gasteria, Puya, Rhododendron* leaf; **e** or **g** for *Primula* leaf; **g** or **i** for *Aucuba* leaf. *Helianthus annuus* shows **h** distinctly in the cuticle of the stem as well as in the cell contents of the epidermis, with an unstained cellulose inner layer to the outer cell wall.

The fact that this acid reaction is given either only or more strongly by the outermost layer of the cuticle would seem to indicate the influence of acid pectic substances rather than fatty acids (cp. **Lamatière**, cited by **Lee** and **Priestley** 1924). The cutinogenic fatty acids are still obscure but for indicator dyes pectic reactions may be more important.

(e) *Equilibrium Points.* Apart from the observations of a series of possible isoelectric points for the artificial cell membrane investigated by **Mac Dougal** and **Moravek**, the only case of an apparent isoelectric point connected with the wall structure is that recorded by **Lloyd** and **Úlehla**, see above p. 347. There an agar-like composition of the wall seems probable and the case does not apply to higher plants.

Nevertheless, the presence of phosphatides and pectic substances acting as buffers within more or less definite ranges of pH might lead to the so-called isoelectric point phenomena being controlled by the wall substances as well as by the buffer content of the vacuolar sap. This might account for the odd 3 % in acid fluids and the 25 % in alkaline fluids found by **Youden** and **Denny** as the residual buffer effect of the potato tissue, after deducting the effect due to water extractable substances which were presumably the buffer systems of the sap.

(f) *Membrane Buffering: Wall, Cytoplasm and Sap.* We are now in a position to envisage the plant cell as a whole, made up of three parts, wall, cytoplasm and sap, and to consider the pH phenomena of this system.

The sap we have found to be an aqueous solution containing a variety of buffer systems sometimes very weak as in the sunflower and at others of varying buffer values as in the broad bean and potato, leading up to the strongly buffered saps of some succulents. Carbonic acid may, and probably does play a part in determining the actual pH of all but the more acid of these systems.

The cytoplasm we have found to be a strongly buffered colloidal mixture mainly of ampholytes, showing probably a very limited range of actual pH (5·0—6·2). This strong buffer capacity in itself would make the cytoplasmic layer an effectively buffering membrane so far as strong acids and alkalies are concerned; but the passage of weak acids, notably carbonic acid, and to a lesser extent the passage of weak alkalies would not be effected to the same extent. The colloidal structure of this membrane with its accompanying surface-charge phenomena would strengthen considerably its effectiveness as a buffering membrane.

The wall we have found to vary considerably in composition. The suberised wall as a waterproof layer does not permit of buffer action. The lignified wall with its many thinner unlignified pits and other portions may leave the buffer phenomena of any particular cell similar to those of unlignified cells. The cutinised wall may be pectic in nature and more or less similar to normal parenchymatous cells[1]. This would appear to be the case with the exine of pollen grains, if the all-over penetration of acid fluids noted by Lloyd and Úlehla (1926) be general. Finally it would seem that the ordinary typical cellulosic wall may be dominated by its pectic and or phosphatide content, so that in it we may get membrane buffering somewhat like that of the cytoplasm, but not so strong[1].

The vacuolar sap is, therefore, surrounded by at least one very effective buffering layer and possibly by a second rather less effective membrane capable of similar buffer action. One is not now surprised that, while the pH of the cytoplasm and that of the 'cellulosic' wall appear to be relatively constant (varying only within narrow limits), the pH of the sap is affected very little by the external environment, (excepting always the readily permeating carbon dioxide), but it is affected very largely by the metabolism of the cell of which it is the innermost part.

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[1] Rouppert (1926) detects Liesegang zone phenomena in the stinging hair of the nettle which may indicate an unequal distribution of these substances in the cell wall.

CHAPTER XIX

BUFFERS AND BUFFER-INDEXES IN PLANTS

(a) Buffering Substances; (b) Sources; (c) Quantities; (d) Buffer Indexes; (e) Buffer Index Curves; (f) Buffer Complexes; (g) R. I. M. and Buffers.

Carbonic acid in general and organic acids in many cases are the chief agents in the increase of [H] in plant tissues. The removal of carbonic acid, as carbon dioxide, either by gaseous diffusion or by photosynthetic utilisation would appear to be the chief method of decreasing the [H˙] in plant tissues. Few plant cells actually produce alkali in metabolism, although trimethylamine gives a characteristic odour to quite a number of plants (see CZAPEK I, p. 780).

Our detailed knowledge of acid-producing metabolism is so meagre that it would seem better to proceed by small steps and determine first of all the actual acids produced, together with the quantities and the effects of the occurrence of these quantities upon the life of the cell. In this way we arrive at the subject of this chapter. The qualitative identification and quantitative determination of the buffering substances in plant juices which was attempted for succulents by HEMPEL (1917) has been extended in this Department to ordinary plants by MISS S. H. MARTIN (see Chapters XII—XIII), by C. T. INGOLD (see Chapter XIV) and J. I. ARMSTRONG (see below). By means of the analytical schemes given in Appendices I and II (pp. 379, 381) there is no reason why our knowledge of this part of botanical physiology should not be extended rapidly, so that sound generalisation may become possible. HEMPEL'S method, applied only to *Rochea* and *Cotyledon obvallata*, was essentially that of quantitative comparison of known mixtures with the natural sap. It might be useful still as a check.

(a) *Buffering Substances.* The following acids have been identified by MARTIN (p. 208) and INGOLD (p. 291) and ARMSTRONG (p. 361) as forming, with bases, buffer systems in plant juices:

Acid	pK	Type of Buffer System	Steps
Carbonic	6·33 (6·52)	H_2CO_3 — $KHCO_3$	1st
	10·22	$KHCO_3$ — K_2CO_3	2nd
Phosphoric . . .	2·11	H_3PO_4 — KH_2PO_4 .	1st
	7·16	KH_2PO_4 — K_2HPO_4	2nd
	12·66	K_2HPO_4 K_3PO_4	3rd
Citric	pK′ 3·08	$CH_2 \cdot COOH$ $\vert$ $C(OH) \cdot COOH$ — $KH_2C_6H_5O_7$ $\vert$ $CH_2 \cdot COOH$	1st
	4·39	$KH_2C_6H_5O_7$ — $K_2HC_6H_5O_7$	2nd
	5·49	$K_2HC_6H_5O_7$ — $K_3C_6H_5O_7$	3rd
Malic	3·48	$CHOH \cdot COOH$ $\vert$ $CH_2 \cdot COOH$ — $KHC_4H_4O_5$	1st
	5·11	$KHC_4H_4O_5$ — $K_2C_4H_4O_5$	2nd
Tartaric (dihydroxysuccinic)	3·0	$CHOH \cdot COOH$ $\vert$ — $KHC_4H_4O_6$ $CHOH \cdot COOH$	1st
	4·39	$KHC_4H_4O_6$ — $K_2C_4H_4O_6$	2nd
Oxalic	1·42	$COOH$ $\vert$ $COOH$ — KHC_2O_4	1st
	4·39	KHC_2O_4 $K_2C_2O_4$	2nd
Asparagin	pKA 2·08	$NH_2 \cdot CO \cdot CH_2 \cdot CH(NH_2) \cdot COOH$ $NH_2 \cdot CO \cdot CH_2 \cdot CH(NH_2) \cdot COOK$	monobasic

Amongst other organic acids which may be found to form effective buffer systems in plants are:

Acid	pK[1]	Type of Buffer System	Steps
Malonic	2·80	$CH_2(COOH)_2$ $KHC_3H_2O_4$	1st
	5·68	$KHC_3H_2O_4$ $K_2C_3H_2O_4$	2nd
Succinic	4·18	$CH_2 \cdot COOH$ $\vert$ $CH_2 \cdot COOH$ $KHC_4H_4O_4$	1st
	5·57	$KHC_4H_4O_4$ — $K_2C_4H_4O_4$	2nd
Pyrotartaric . . . (methyl-succinic)	4·1	$CH_3 \cdot CH \cdot COOH$ $\vert$ CH_2COOH — $KHC_5H_6O_4$	1st
	5·63	$KHC_5H_6O_4$ — $K_2C_5H_6O_4$	2nd
Glycollic	3·82	$OH_2 \cdot CH \cdot COOH$ $OH \cdot CH_2 \cdot COOK$	monobasic

The bases associated with these acids would appear to be those usually found in plants, namely potassium, calcium, magnesium. HEMPEL found these, and also aluminium and sodium in *Rochea* and *Cotyledon obvallata*.

(b) *Sources*. The source of the bases is clearly the soil solution and the same is true of the phosphoric acid. All these would be absorbed by the plant as soluble salts, nitrates, chlorides, sulphates and phosphates.

The sources of the organic acids are still obscure but a probable origin of some or all of these may be traced in the abnormal metabolism of the acid succulents concerning which so much work has been done. In general they may be described as arising as intermediate products in the oxidation processes which yield carbonic acid, but the factors which carry that process to the oxalic acid stage in some cases and only to the citric or malic acid stage in other cases are still obscure. Hypotheses are easy, and the facts are too complex for anything but a systematic analysis.

(c) *Quantities*. HEMPEL (1917 p. 63) found that in the region of pH 5·5 the quantity of acids in the various plants examined did not differ greatly one from another and that an increasing divergence in acid content was found with a natural increase in [H] towards pH 4 in the saps. These acid contents can be calculated from HEMPEL's data as, at pH 4·0, *Kleinia* ·12 N, *Rochea* ·11 N, *Crassula* ·09 N. These data were obtained by titration to the litmus point and represent molar concentrations in the region of ·24 M to ·08 M of malic acid salts or salts of similar dibasic acids with low dissociation constants. Considering only monovalent bases and taking *Kleinia* as containing equal parts of free dibasic acid and acid-salt we get ·08 M, while with equal parts of acid-salt and neutral salt we get ·24 M.[1]) The presence of calcium and magnesium complicates the real conditions and the actual concentration can be determined only by specific quantitative analysis of the juice, or titration to the litmus point of a solution containing only free acid which does not appear to occur in acid succulents (cp. p. 292 sqq.). HEMPEL (ibid. p. 62) gives data from which the maximum possible phosphate (H_3PO_4) content of *Rochea* juice can be calculated as ·0023 M approximately.

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[1]) The ratio of the various salts is determined by the pH of the solution, see p. 71.

Quantitative or even qualitative determinations of specific organic acids in plant juices are very rare in botanical literature[1]), but useful analytical data can be found in Tibbles (1912) and similar books of reference. Some of these for fresh foods may be quoted as giving an idea of the distribution and concentrations of organic acids and phosphates which occur in parts of the plant.

Plant	*Juices*	*Phosphate*	*Organic Acid*
Vicia faba p. 263	stem	·0134 M	·005 M bicarbonate ·0211 M oxalate ·011 M malate
Helianthus annuus p. 218 sqq.	hypocotyl	·005 ·007 M	
	stem	·0044 ·0050 M	
	root	·0033 - ·0034 M	
Solanum tuberosum p. 291	tuber	·0037 ·0069 M average ·005 M	·0057 M citrate
Succulents p. 296	shoot		·106 ·016 M malate
Rochea falcata p. 296	leaves	0023 M	·106 M malate
Bryophyllum p. 296	leaves	...	·05 ·073 M malate (see also Table XII)

Plant	*Part*	*Phosphate*	*Organic Acids*
cereals	seeds	·06 ·09 M	Data from "Tibbles". molar conc. calc.
potato	tuber	·003 M	
carrot	root	·002 M	malate
pulses	seeds	·02 ·04 M	
asparagus. . . .	tops	-	asparagin and malate
onion, etc. . . .	bulbs	present	acetate, malate
lettuce	leaves	·001 M	oxalate, malate
fungi.	fresh	·003 ·021 M	citrate, malate, oxalate
	dried	·03 ·20 M	fumarate (see also Table XII)
fruits.	fresh	present	various see below

In fruits, malates occur in the apple, pear, quince, cherry, white and red currants, blackberry, pineapple and rhubarb;

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1) Abbott (1923) found an inverse relation between phosphate content and [H˙] and suggested phosphates as important buffers in plants.

oxalates in tomato, plum, gooseberry, strawberry, and raspberry; citrates and free citric acid in lime, lemon, orange, quince, tomato, raspberry and gooseberry; tartaric and racemic acids in grapes.

(d) *Buffer Indexes.* Apart from those already given in this volume, see Chapters XII—XV, there are few or no records of the β values for plant fluids, but in some cases data have been given by previous authors which enable this useful value to be calculated for the fluids which they investigated. The values for succulents, over rather wide ranges, have been given and discussed in Chapter XV.

β Values. The following Tables (pp. 358—361) give the β values for certain acids and for some plants calculated from the data given by the authors mentioned.

These data for fungi will be considered fully in a forthcoming contribution to *Protoplasma.*

(e) *Buffer Index Curves.* Examined in the form of tabulated data, buffer indexes are difficult for biologists, who can as a rule follow the analysis of such phenomena better when the data are presented graphically. Two figures have, therefore, been prepared, showing how the buffering capacity or buffer index of various acid-plus-salt systems varies throughout the pH range; figure 27 gives the values for a concentration of one-tenth molar; figure 28 gives the corresponding values of one or two systems for one-hundredth molar, together with lemon juice which is about ·3 molar; this latter is separately graduated on the right. All the curves in figure 28 except lemon juice could, therefore, be inserted as very much flattened curves in the basal section (0 to ·01 β) of figure 27, but by increasing the vertical scale we can present these 0·01 molar (·001 --·010 β) curves more effectively.

These curves are calculated to 0·2 pH changes from the same sources as in Table XI. The malate, oxalate, tartrate, amino-acetate and succinate data were obtained in this Department by Mr. C. T. Ingold using a quinhydrone electrode on 0·025 M solutions.

Characteristics of β Curves. — In order to make full use of these curves and to take advantage of the points in which a β curve is clearer than a titration curve, the characteristics of these buffer index curves must be studied carefully. The main features to be noted are (a) the position and (b) the duration of the maximum in relation to the pH range; (c) the position and (d) the

Table XI

Average **0·1** *Molar Buffer Indexes* (β calculated from data)

pH RANGES

Acid	1—2	2—3	3—4	4—5	5—6	6—7	7—8	8—9	pKa	T°	Source of Data
Boric	—	—	—	—	—	—	·008	·032	9·18	25	Clark
Carbonic 1st	—	—	—	—	·02	·06	·016	—	6·52	18	Kolthoff
do. 2nd	—	—	—	—	—	—	—	—	10·22	18	do.
Phosphoric 1st	—	·048	·010	·004	—	—	—	—	1·96	25	Clark
						·057					
do. 2nd	—	—	—	·004	·010	·048	·034	·008	6·8	25	do.
Clark's Phosphate Buffers	—	—	—	—	·2 ←	·575	→ ·2	—	—	—	do.
Hydrochloric	·081	·017	·002	—	—	—	—	—	—	—	do.
				·057							
Acetic	—	—	·018	·052	·025	·003	·001	—	4·73	25	do.
		2·5-3·0									
Citric 1st	—	·070	·0703	·0805	—	—	—	—	3·08	25	Sørrensen's Buffer Mixture
do. 2nd	—	—	·0703	·0805	·0645	—	—	—	4·39	18	do.
						6·0-6·6					
do. 3rd	—	—	—	·0805	·0645	·035	—	—	5·49	18	do.
Malic	—	·061	·061	—	—	—	—	—	3·48	25	Ingold
do.	—	—	·061	·063	·037	·009	—	—	5·11	18	do.
Oxalic 1st	—	·061	·048	·040	—	—	—	—	1·42	25	do.
do. 2nd	—	—	·048	·040	·011	—	—	—	4·39	18	do.
Succinic 1st	—	—	·038	·072	—	—	—	—	4·18	25	do.
							7·0-7·5				
do. 2nd	—	—	—	·072	·065	·021	·0064	—	5·57	18	do.
Tartaric 1st	—	·080	·077	—	—	—	—	—	3·01	25	do.
do. 2nd	—	—	·077	·055	·012	·003	—	—	4·39	18	do.

Table XI Continued

Average 0·1 *Molar Buffer Indexes* (β calculated from data)

pH RANGES

			3—4	4—5	5—6	6—7	7—8	8—9			Source of Data
Bryophyllum calycinum cloudy day.	—	—	—	·0879	·0488	·0146	·0146	—			Gustafson
Bryophyllum calycinum sunny day	—	—	—	·0197	·0146	·0049	·0057	—			Am. J. Bot. XI, 1924
Zea mais leaves	—	—	—	—	—	·0147		—			do.
Cucurbita maxima leaves.	—	—	—	—	—	—	·0142				do.
do. do. stem .	—	—	—	—	—	—	·0076				do.
Pyrus malus juice from "Greening" apples . .	—	—	·0328	·020	·015		·0030				Youden & Denny 1926
Helianthus annuus, hypocotyl	—	—	—	—	·0020		—	—			Martin 1927
Helianthus annuus, stem .	—	—	—	—	·0015		—	—			do.
do. root .	—	—	—	—	·0010		—	—			do.
					0037	·005					
Vicia faba, stem	—	—	—	—	·0125		—	—			Martin 1928
do. root	—	—	—	—	·0075		—	—			do.
1 % Tuberin	—	—	·0033	·0011	·0006	·0008	·0011	—			Cohn ex Osborne
1 % Gluten	—	—	·0017	·0010	·0006	·00025	·0002	—			Osborne 1924
1 % isoelectric cryst. egg albumin	—	—	·004	·0026	·0014	·0008	·0005	—			Loeb 1924
1 % Gelatin	—	—	·006	·0034	·0019	·0011	·0004	—			do
1 % Casein	—	—	·0058	—	—	·004	·002	—			do.
			3·3—4				7-7·7				
2 % Asparagin	—	—	·0071	·0019	·0002	·0019	·0067	—			C. T. Ingold 1928

Table XI Continued

Buffer Indexes (β calculated)

	1—2	2—3	3—4	4—5	5—6	6—7	7—8	8—9		Source of Data
Hordeum (barley tops) . .	—	—	—	—		·0122				Haas 1920
Zea mais (corn tops-limed)	—	—	—	—	·022	·0119	·006	—		do.
Avena (oat tops-limed) .	—	—	—	—		·0206		—		do.
Trifolium (medium red clover)										
do. tops	—	—	—	—	—	·0086	·0115	·0230	—	do.
do. leaves	—	—	—	—	—	·0222	·0261	—		do.
do. stem and petioles	—	—	—	—	—	·0058	·0097	·0161	—	do.
do. roots.	—	—	—	—	—	·0060	·0161	·0323		do.
Medicago (alfalfa tops) . .	—	—	—	—	—	·0132		·0208		do.
Lupines (yellow lupin, unlimed tops)	—	—	—	—		·0110				do.
Reserve Tissues	pH 3·0		4·0		5·0		6·0		7·0	
Potato	—	—		·026		·0105		·0079		Ingold (1929)
Turnip.	—	—	·0145	·0124	·0122	·0083	·0062	·005	·004	Ingold (unpub.)
Onion	—	—	·0120	·0106	·0092	·0070	·0046	·0040	·0038	do.
Beetroot	—	—	·0085	·0071	·0058	·0046	·0035	·0023	·0026	do.
Fungi	see Table XII									Armstrong (unpub.)

Table XII

Fungus species	pH of Sap	β Values for pH 4—5	β Values for pH 5—6	β Values for pH 6—7	Maximum region	Maximum β value	Analytical notes
Lactarius torminosus .	6·3	—	0·02296	0·00191	5—6	0·02296	by J. I. ARMSTRONG (unpub.)
Coprinus atramentaruis	6·9	0·00329	0·00427	0·00392	5—6	0·00427	Q. U. B.
Polyporus sulphureus .	6·7	0·00609	0·00623	0·0035	5—6	0·00623	
Armillaria mellea . . .	6·3	0·0275	0·01236	—	4—5	0·0275	
Coprinus micaceus. . .	6·5	0·0102	0·0056	0·0037	4—5	0·0102	0·00614 M phosphate 0·0284 M oxalate 0·0187 M citrate 0·0025 M malate 0·0005 M tartrate
Lycoperdon pyriforme .	6·5	0·00745	0·00414	0·00336	4—5	0·00745	
Lactarius blennius . .	6·0	0·0059	0·00221	0·00191	4—5	0·0059	
Polystictus versicolor .	6·7	0·00185	0·00079	0·00095	4—5	0·00185	
Hypholoma fasciculare.	5·5	0·00922	0·00575	0·00369	4—5	0·00922	0·00397 M phosphate
Collybia velutipes . .	6·2	0·0257	0·0179	0·0123	4—5	0·0257	0·0101 M phosphate 0.014M citrate; 0·0228 M malate Buffer Complex

β for pH ranges . . .	3·5—4·0	4·0—4·5	4·5—5·0	5·0—5·5	5·5—6·0	6·0—6·5	6·5—7·0	7·0—7·5	
Collybia velutipes . . .	0·0273	0·0262	0·0252	0·021	0·0148	0·0134	0·0112	0·0092	For expressed sap
	—	0·0255	0·0260	0·0217	0·0158	0·0119	0·0095		For Buffer complex as above

steepness of the slope on one or both sides of the maximum. Some organic acids pass almost directly from their own maxima to the final rapid rise below pH 2·5 in which the so-called buffer value of a water plus strong acid system takes effect (see figs. 9—10); in these cases there is no fall on the more acid side of the maximum (cp. the acetate and oxalate curves with the malate and tartrate curves, fig. 27).

Phosphate. — This curve rises to a maximum at pH 6·8, falls towards pH 7·5 and to zero about pH 4·5, rising again slightly in the region of H_3PO_4—KH_2PO_4 buffering (pH 2—4), see also figure 12.

Bicarbonate -carbonic acid. — This behaves in a very similar way to the phosphate curve, but the values in the zone pH 7·8 are lower, and there is no second rise on the acid side, because below pH 5·2 all the carbonic acid is free. The maximum occurs about pH 6·5.

Oxalate. — This system is mainly acid-oxalate plus neutral oxalate, with the curve very low in pH 5—6, rising in pH 4—5, to a maximum about pH 3·9, falling towards pH 3·0 and rising again to its own *primary* maximum which passes into the strong acid-water rise.

Malate. — This is again an acid salt + neutral salt and acid salt + acid system, complicated in this case by the much closer approach of the pK values for the two maxima. This closeness results in a practically horizontal curve from pH 2·5 to pH 5·0, with a steep drop between pH 5 and pH 6·0, passing to zero about pH 7·0.

Tartrate. — This resembles the malate curve closely, but has a higher maximum and drops in the zone pH 4—5, instead of in pH 5—6.

Citrate. — As a tribasic acid with fairly well separated pK points, this forms buffer systems with a primary maximum about pH 4·6 and two secondary maxima, one for the first step about pH 3·1 and another for the third step about pH 5·4. This curve rises steeply through the range pH 5·6—6·6.

Succinate. — As that of a dibasic acid with pK values near together this curve shows the flatness of malate and tartrate curves, but it resembles the citrate curve between pH 4 and pH 7. Succinic acid is not very common in plants, so that no more need be said.

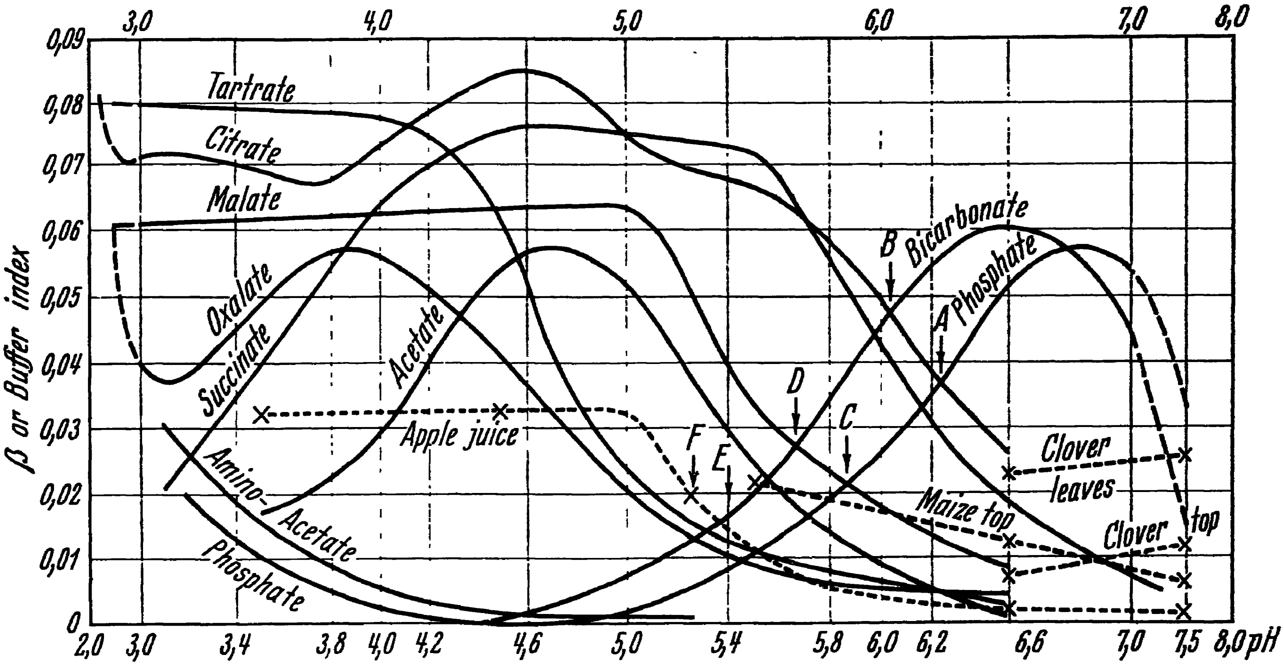

Fig. 27. Buffer Index Curves for 0·1 molar solutions and for apple juice, maize tops, clover leaves and tops.

Acetate. — As that of a monobasic acid the acetate curve reaches its maximum at $\beta = \cdot 0575$, which occurs about pH 4·7, with a fall on either side which is more marked in pH 4·0 - 4·7. The fall in pH 5—6 is similar to that of the malate curve, but the acetate curve resembles in general that of succinate.

The β curves having been characterised in this way, it becomes clear that *if* there be only *one* effective buffer system in a sap, this system can be identified qualitatively by constructing a β curve throughout the range pH 3·0—pH 7·0. Further, since the β values are directly proportional to the molar concentration in a simple buffer system (see[1]) p. 289), it becomes possible to identify a *single* buffer system in sap quantitatively as well as qualitatively. As an example, the apple juice curve in fig. 27 from the data given by YOUDEN and DENNY is clearly that of a 0·05 molar solution of malates, since it is the same shape with the same position and has throughout about half the values of a 0·01 molar malate curve[1]). Similar quantitative identifications have already been given for *Bryophyllum* p. 296 and other succulents p. 296. Sunflower sap (fig. 28) should be compared with the 0·01 M phosphate curve in the same figure. The lower concentration of phosphate naturally flattens the curves for this sap

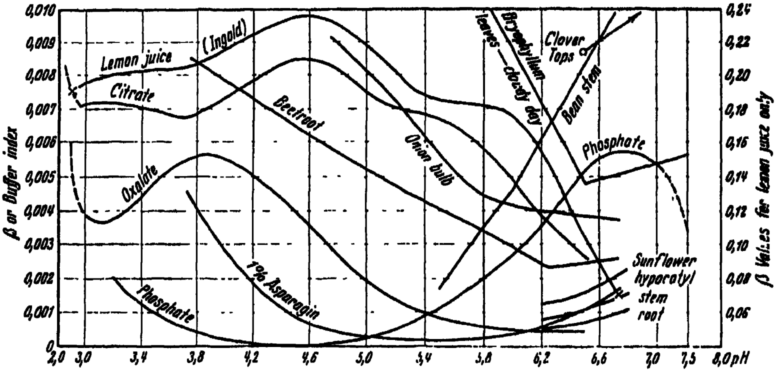

Fig. 28. Buffer Index Curves for 0·01 molar solutions and for various plant juices.

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[1]) The quality or nature of the buffer determines the shape of the β-curve and its position on the pH scale, but the quantity of buffer determines the actual β values, compare ·01 citrate and lemon juice in figure 28.

as compared with 0·01 molar phosphate. In the same way the curve for the bean stem sap is much steeper than 0·010 molar phosphate, but a 0·015 molar phosphate curve *on the same vertical scale* would be nearly coincident with that of the bean stem, and the actual concentration found was 0·0134 M phosphate.

(f) *Buffer Complexes.* In natural plant saps it frequently happens that, although there may be one main buffer system which is effective in the natural range of pH for that particular sap, more than one buffer system occurs. Then it becomes more difficult to analyse the β curve of the sap; for example, clover tops and leaves and maize tops (fig. 27, data from HAAS 1920) are apparently not dominated by any one buffer system even in the range of pH recorded.

The possibilities can be indicated by a few simple examples from figure 27.

Phosphate plus Citrate. — Equimolar concentrations of these two systems would give a flat curve, rather like that of maize tops; since the additive effects in the region between pH 5·0 and pH 6·8 would practically eliminate the dip at A (cp. also fig. 21, Chapter XIV). Unequal concentrations of the two systems would result in a straight β curve with a stronger upward tilt towards the acid side if the citrate were greater and a horizontal line or an upward tilt to the neutral side if the phosphate were greater, rather like those for clover tops and leaves (fig. 27).

Phosphate plus Malate. — Equimolar concentrations of these two systems would show a slightly lower buffer index about pH 5·8 (C) if the determinations were made for 0·2 steps in pH but determinations for 0·5 or unit pH would result in only a very slight depression or none at all, in the range pH 5—6. With more phosphate than malate this dip would be deepened for unit pH steps and moved to the left towards E for 0·2 steps in pH. With more malate than phosphate the β curve would resemble a citrate plus phosphate curve in the zone pH 4·0—7·0 and would show no decided fall in pH 3—4. A citrate plus phosphate system would be very similar, since the phosphate rise in pH 3—4 compensates more or less the drop in the citrate β values in that region.

Phosphate plus Oxalate. — In equimolecular concentrations these two systems would show a decided dip in pH 5—6, about E (fig. 27) with 0·2 pH steps. Below pH 5·0 this complex would give almost a pure oxalate β curve, rising towards pH 4·0 and falling towards pH 3·0.

With phosphates plus organic acids the β curve in all cases below pH 5·0 is practically that of the organic acid + salt system. It thus becomes a question of distinguishing between the β curves of organic acids with quantities at first unknown.

Single organic acid + salt Systems. — If the titration be carried out very carefully to 0·2 or 0·5 pH changes, the *form* of the curve in the unknown solution can be used. After a quantitative phosphate determination the rise of phosphate in pH 3—4 can be deducted, and then it becomes possible from the form of the curve to identify tentatively the acid present, assuming it to be a system with only one organic acid. The β curve for each acid has quite a distinct form when the position of the curve on the pH scale is taken into consideration. For comparison with figure 27, the vertical scale should be in strict proportion, thus if the known β values range from ·001 to ·009 instead of ·01 to ·09 they should be plotted so that ·009 extends vertically as high as the ·09 of the figure 27, similarly values of ·005 to ·05 should be plotted so that ·005 is near the present ·01 and ·04 near the present ·08.

Mixed organic acid + salt Systems. — When there is more than one organic acid present, analysis of the buffer index curve becomes more difficult. With titration to 0·2 or 0·5 pH changes, the presence of oxalate (or succinate) can be suspected when there is a fall in pH 3·0—3·5. Malate, citrate and tartrate in mixture with anything less than equimolecular concentration of phosphate are scarcely distinguishable. In particular, a mixture of malate and citrate gives a curve which is difficult to distinguish from either of these alone. The flatness of the malate in pH 2·5—5·0 and the dip of the citrate about pH 3·5—4·0 may show in simple systems, and if something intermediate is obtained mixtures may be suspected. Quantitative analysis must be carried out before the curves of such systems can be regarded as explained[1]).

Tartrate is very similar to malate and citrate, while succinate and acetate are very similar to oxalate but with maxima in pH 4—5, instead of in pH 3—4. These other three acids are not known to be widely distributed in plants, and can be identified fairly easily.

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[1]) The buffer values in a mixed system are directly additive even with asparagin present. (C. T. INGOLD, unpublished work.)

Other Complexes. — In addition to these simple systems the bicarbonate-carbonic acid system is practically always present to some extent when the natural pH is above pH 5·6, and mixtures of organic acids in various proportions would appear to occur rather more frequently than do single organic acids. In such cases quantitative analyses of the sap must be used in order to analyse the buffer index curve of the natural sap. This has been done more or less successfully for the sunflower and the broad bean (Martin), the potato (Ingold) and certain fungi (Armstrong, see Table XII).

As an example of a mixed buffer system we can take the β curve of the onion bulb given in fig. 28. A pure citrate system with $\beta = \cdot 0085$ between pH 3·5—4·0 would show a β curve *above* the 0·01 Molar level throughout. The depression of the onion curve below the citrate curve in the pH 5—6 zone indicates the presence of a system with lower than citrate values between pH 5 and pH 6. This depression might be due in part at least to the phosphate and/or bicarbonate which is indicated by the upward displacement of the ‚citrate-like‘ onion curve in the pH 6—7 zone; but in that case the values in the pH 5 – 6 zone would be additive for phosphate and citrate, and the depression should be smoothed out. On the other hand a malate, tartrate or acetate system added to the citrate plus phosphate system would tend to give the depression observed in the pH 5 - 6 zone. If the titration had been carried into the pH 3 - 4 zone there would have been no difficulty; acetate would show a fall while malate and tartrate would displace the citrate curve upwards. As it is we can *suggest* acetate because, according to Tibbles, acetate and citrate have actually been found in the onion group of vegetables (see p. 356). Quantitative analyses are required to confirm these suggested possibilities, especially in view of Ingold's smooth curve for the onion bulb (see p. 360).

The curve for other plant saps can be tentatively analysed in a similar manner. The smoothly rising curves obtained by C. T. Ingold for carrot, onion and beetroot (see p. 360) indicate more complicated buffer complexes; the rise from pH 4 –5 to pH 3 4 suggesting oxalate or tartrate, and the slight rise from pH 6·0—6·5 to pH 6·5—7·0 suggesting phosphate in the beetroot (see fig. 28).

Buffer Complexes and Natural pH Values. — An examination of fig. 27 shows that practically all the β curves cross between pH 5 and pH 6·2. In the absence of abnormal metabolism this is the range of natural pH in plant cells[1]) and apparently also in cytoplasm. The reason for this should now be clear. Any movement of the pH of the sap (or cytoplasm), either in the direction of greater acidity or in that of lesser acidity, is met by increasing buffer capacity of the buffer complex present[2]). Such movement of the natural pH will, therefore, be retarded to a degree corresponding to the concentration of the buffer system and to the extent (within limits) of the pH swing. The natural pH tends to be in the minimal β zone or the valley of the β curve of the particular buffer complex present, that is at A pH 6·2, or between that and F on the pH scale[3]). In the presence of abnormal acid metabolism the natural pH will be below pH 5·2.

Buffer Complexes and Isoelectric Points. — Almost exactly the same arguments apply to many of the phenomena where the internal buffer systems have an opportunity, during experimental manipulation, of passing into an external fluid. The so-called isoelectric points would appear to be equilibrium zones at the minimal β values of the buffer complexes present and passing outwards into the external fluids possibly at different rates. The variation in the rate of outward flow would account for some of the variation in the values for the equilibrium points recorded.

Buffer Complexes and Disease. Ingold (p. 288) found no correlation between immunity and the buffer index values of potato sap (cp. Kopaczewski 1928 for serum). Campbell (1918) found that extra-radicate injection of weak solutions of tartaric, citric and malic acids rendered cultivated apples immune to *Oidium farinosum* (*B. leucotricha*) and to certain insects. This effect might be due either to a change in actual pH or to a change in buffer capacity.

The whole field of the relation of the internal pH and buffer values to disease has still to be investigated. External pH effects

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1) Algae and Fungi are exceptional, see note 2).

2) This is not found in the fungi examined, to any marked extent, but the bicarbonate rise is there omitted.

3) Compare figure 19.

on the disease organism would appear to have little importance. The important factors are obviously not the residual pH values of expressed sap, but the actual pH of the phases of the living cells of host and parasite, and the buffer values of these phases in relation to the effect produced by the disease. Increase of internal $[H^{\cdot}]$ has been noted in a number of parasitic diseases. This phenomenon was observed by the writer many years ago in the case of leaf-curl of potato, and the results then obtained led to the adoption of the R.I.M. for a general survey.

The possible connection of pH and/or buffer values with infection and immunity appears to be complex. The buffer value may have no effect in certain pH ranges, but quite a distinct effect in higher or lower ranges of pH. The whole field is open for survey and the investigations of the effects of external pH variations which have been made are summarised in Mevius (1927).

(g) *R.I.M. and Buffers.* A close study of the R. I. M. indicator series (p. 49) will show that it is almost continuous; 6·8—6·4, 6·8—6·2, 6·2 ca., 6·2—5·9, 5·9 ca., 5·9—5·6, 5·6 ca.; (5·6—4·8); 5·2—4·8, 5·2—4·0, 4·4 ca., 4·4—4·0, 4·0 ca.; $<$ 3·4. The bridging range d 5·6—4·8, however, does not occur to any extent in our recorded observations and this gives a second break namely between 5·6 and 5·2, as well as between 4·0 and $<$ 3·4. The lower break does not appear to be important in normal plant tissues but another indicator may be required in order to cover the 5·6—5·2 break. We might lower and raise the MR changes to pH 5·3 or pH 5·4 but the orange zone of MR is a perplexing region of uncertainty and all these tints are better taken as indeterminate. The numerous records of the ranges c (5·6 ca.) and e (5·2—4·8) and the very few records of d (see Table IV, Chapter XIII) must mean either (1) that the interpretation of the MR tints is faulty or (2) that when reaction swings take place they pass rapidly across the range pH 5·6—5·2. A possible explanation of the phenomenon is suggested by the general form of the buffer curves, where the buffers are phosphate and oxalate systems. The former steadies or buffers the reaction in the natural range pH 6·2—5·6, while the latter and allied systems buffer the reaction between pH 5 and pH 3. Both systems reach a minimum between pH 5·6 and pH 5; any factor which tends to changes the pH of the fluid will, therefore, be most effective in that range; with the interesting result that the natural pH of the fluid will seldom remain in that range but pass either to the acid range below pH 5·2 or to the relatively alkaline range of pH 5·6 ca., or above. Where citric or malic acid or both occur the range d is more likely to be observed as these form buffer systems active in the range pH 5·6—5·2. Malic acid is found in *Vicia faba*, and so is the range d, see Tables IV, V, VI, Chapter XIII; compare also Table XXV, with Table XXIV in the same chapter, where a pure phosphate system has not the same buffer effect against CO_2 that is possessed by the malate-containing bean sap.

We come here upon certain fundamentally different types of cell metabolism.

The first type is that of the normal parenchymatous cells or tissues in plants. In this first type the fluids are only very slightly acid pH 5·6—6·2, and fluctuations within this range are probably governed mainly by the carbon dioxide content and controlled by the phosphate buffer system and/or the bicarbonate-carbonic acid buffer system. These would appear to be the conditions under which normal respiration, carbon assimilation, initial wound reactions (cp. p. 266) and turgidity changes proceed.

The second type is that which results (1) in differentiation of tissues, within the plant; acid epidermis and dermatogen, lignification, suberisation, cuticularisation, development of xylem, endodermis, cork, and root tips; and (2) in a differentiation of types of metabolism as characterising certain groups of families, isolated families, or isolated genera within normal families. In this second type the carbohydrate katabolism would appear to be not the normal process of complete oxidation to carbon dioxide and water, but an incomplete oxidation resulting in the production of organic acids either directly or indirectly as by-products of the main metabolism.

This type of metabolism occurs in normal plants when the carbohydrates are being used in the formation of lignin, suberin and cutin. Whether the fatty acids directly concerned give a true virage with indicators, as do acetic to pelargonic and the lower fatty acids or whether they take up only the neutral form of some indicator dyes as does oleic acid, in either case this metabolism would appear to be associated with the production of acids which do give a true virage with most indicator dyes. Differentiation of walls is, therefore, accompanied by this acid type of metabolism.

This type of metabolism also appears in special groups of families or in isolated genera where the normal complete oxidation of carbohydrates does not occur even in the parenchymatous tissues. The acidity in this case may be due to various organic acids, oxalic in Polygonaceae, oxalic and malic in Aizoaceae, malic in cacti and some Rosaceae, isomalic in Crassulaceae, etc.

The differentiation of these two types of relatively alkaline and relatively acid metabolism was one of the first points in the R.I.M. general survey. It can be seen using only the one indicator methyl red, and was noted but not developed by ROHDE in his 1917 contribution.

The fluctuation in reaction of tissues with the acid type of metabolism would appear to be governed by the type of acid produced and the concentration of that acid and not to any significant extent by the carbonic acid content. The reaction in these cases would be controlled by an organic-acid plus salt buffer system or a mixture of such systems. The scheme of analysis given in Appendix II enables us to determine the identity of the main buffer systems and to get some quantitative ideas of the parts they play within the tissues examined.

CHAPTER XX

PROBLEMS RE-STATED

Having brought most of the known facts together, we are now in a position to consider how far the problems, which were raised more or less *a priori* in Chapter 1, have been solved.

PROTEINS

These have been found to be more restricted to the cytoplasm and much less effective in the sap and wall than at one time seemed possible. The protein problems are, therefore, confined to the cytoplasm. It has also been found likely that, in the plant cell, the real pH of the cytoplasm does not vary in the living cell beyond the range pH 5·0—6·2. Practically all the experimental investigation of the relation of cytoplasmic phenomena to $[H^{\cdot}]$ has been conducted with external fluids or internal sap known to vary beyond this range. There has been no investigation of cytoplasmic phenomena during which the real pH of the living cytoplasm has been kept under observation, except those of Kuwada and Sakamura on chromosomes. Our knowledge of these phenomena is, therefore, confined to such as are affected by the pH of the fluid in external or internal contact with the cytoplasm.

The physical condition of the cytoplasm has been shown to undergo changes which are possibly related to pH variations induced by external carbonic acid (JACOBS) and these changes are such as would influence strongly the semipermeable properties of the layer. It has been found quite commonly that strong acids do not have this effect, so that we have rather definite knowledge that carbonic acid is an important substance in relation to the cytoplasm. Much more investigation is required before we can be said to know what happens under natural conditions with natural intercellular concentrations of carbon dioxide.

Concerning the stability of cytoplasmic proteins, we know (1) that they are *more or less* stable between pH 5·0 and pH 6·2, (2) that they can endure an external or vacuolar [H˙] of pH 4·8 passing in some cases to pH 4·0 *ca.* or in special cells to pH $<$ 3·4, and (3) that in special cases the external pH may even go below pH 1. We know very little indeed about the actual protein complexes present in the living cytoplasm.

These pH relations apply to the ordinary plant cell and to vacuolated meristem-cells such as occur in cambium, but the more or less non-vacuolated cells of apical meristems are dominated by cytoplasmic buffering and as such are subject to fluctuations beyond the range pH 5·0—6·2 only in the external fluids. In these cells the hydrophilic and swelling properties of colloidal protein may be effective but, in the vacuolated cell, permeability changes would appear to be the only important characteristic affected by [H˙]. The evidence recorded for iso-electric points in plant cells and tissues is in a very large measure concerned either with injury effects at low pH values or with the buffer complexes of the vacuolar sap, within the vacuoles or after passing into the experimental external fluids.

The changes recorded in the physical condition of chromosomes at varying pH values, these values being controlled experimentally by carbonic acid, are of outstanding importance; but much more investigation along similar lines is required before we can generalise upon natural pH values as controlling factors in nuclear phenomena. Degeneration of pollen grains, and other abnormalities connected with nuclear failures may or may not be controlled by the pH of the cytoplasm. A fruitful field of work has been opened up, but we are still just at the gate.

The main outstanding problems in relation to proteins and pH undoubtedly require the investigation, under a carefully controlled carbon dioxide pressure, of the effects of varying the [H˙] *within the natural range* upon the permeability of the cytoplasmic layer to solutes of various kinds. In such investigations attention must be given to the existence and effects of a pH gradient across the cytoplasmic layer and to the direction of such gradient (cp. p. 318).

ENZYMES

The relation between enzymic activity and [H˙] is known for all the chief enzymes; but there appears to be a strong diversity of opinion about this in the case of oxidases and peroxidases which are very important in plant physiology. The causal relations of oxidation and acidity or acidity and oxydase activity require elucidation.

The main problem with enzymes is their detailed distribution *within* the plant. It is definitely known in some cases and it seems probable in many more cases that the distribution of enzymes differs from plant to plant and from organ to organ or tissue to tissue within the same plant; but actual data concerning the variation of enzymic content in different tissues within the same organ are relatively few and are confined almost entirely to phloem fluids and the petals of flowers with colour-producing enzymes.

The fact that invertase and lipase are Class A (acid) enzymes is probably closely related to the apparent production of organic acids and consequent acidification of all or some tissues as germination proceeds, but other similar relations might be traced if we knew more about the distribution of enzymes in the various tissues of ordinary plants.

BUFFERS

The pioneer work of HEMPEL, and the more recent work by HAAS, GUSTAFSON, YOUDEN and DENNY have been utilised in this volume to give some *quantitative* ideas concerning the buffer capacity of plant saps. The recent work of MARTIN, INGOLD and ARMSTRONG in this Department has enabled us to write of buffer systems which are identified both qualitatively and quantitatively.

We know now that carbon dioxide metabolism in respiration and photosynthesis can be and probably is an effective metabolic buffer process in the sap of ordinary plant tissues. We also know that amino-acids and proteins have usually a negligible effect upon the buffer capacity of these saps.

Further, we know in a few cases only the actual buffer complexes present in the parenchymatous tissues (as pressed juice). We can assume the soil as the source of phosphates, and the katabolism which yields carbon dioxide as the source of bicarbonates. This leaves the sources of the organic acids found still a mystery which may be named but not explained by saying that these acids result from an abnormal 'acid metabolism', possibly incomplete oxidation of carbohydrates.

This 'acid metabolism', its origin and its effects, the factors which control the type and quantity of acid produced and those which control the utilisation of the acid are explained to a certain degree for succulents; but even in that case we know more of the utilisation of the acid than of the earlier stages in the process. The elucidation of these points, for acid tissues in normal plants as well as for 'all acid' plants, for the fatty acids of the wall as well as for the simpler organic acids of the sap, forms the main problem to be solved in connection with 'acid metabolism'.

Malates, citrates and oxalates are common in the more acid families or plant organs, while phosphate and bicarbonate systems would appear to be common in ordinary plants but it is unwise to generalise from the three or four known cases, all of which differ one from another. Much more extensive investigation is required on the buffer complexes of various types of plants and plant tissues. The physical chemistry in relation to acidity, even the chemical composition, of the various kinds of cell-walls is still almost a closed book, which from the picture on the cover promises to be very interesting.

SAP, CYTOPLASM AND WALL

The relation of the accumulation of acid and of basic dyes within the cell has been shown to be largely a question of the pH of the *sap*, and further investigation is needed on the possible connection between the pH of the vacuolar sap and the rate of penetration of salts. This clearly is of much greater import in the life of the plant than the rate of penetration of dyes, and will probably vary not only with the pH of the sap but also with the character and concentration of the buffer systems present in the sap (cp. HOAGLAND and DAVIS 1923, see p. 317 above).

We know that there may be a large difference between the pH of the cytoplasm and that of the sap, which difference appears to be maintained by the buffer action of the cytoplasm, either by the whole layer or by the plasma membrane. This membrane buffer effect would appear to be important in relation to the penetrability of external acids and in relation to the maintenance of free acid as a constituent of the vacuolar sap. In succulents, for example, the malic acid produced does not escape from the cell as such and passes out of the non-green cells only after it has, as free malic acid, been converted into carbon dioxide and water.

There is no evidence that light decomposes malates in the same way that it does free malic acid and the buffer effect of malates becomes important in regulating the pH of the sap and, therefore, the amount of malic acid available for decomposition during the day. Similar relations may or may not hold for other changes in sap or in cell-wall. They require investigation.

We begin to get some idea of the possibilities in the indicator colour reactions given by certain cell-wall substances but we have just become aware of the existence of these problems which promise to lead us into the by-paths of fatty acid physical chemistry. Even the precise problems are still obscure; when we have formulated them the way will undoubtedly become clearer.

VARIATIONS IN REACTION

The R.I.M. survey has definitely solved most of the problems of the actual variation in reaction of cell sap from one part to another of the cell; within the same or in similar cells, from time to time, diurnally, seasonally, and during the maturing of the plant or the tissue; in the same tissue from one part to another of the same plant; from plant to plant, species to species, genus to genus, family to family; and to some extent even from group to group.

Other workers have found internal variation or lack of variation with the external medium; variation, of external medium only, in relation to the photosynthetic activities of submerged green plants; internal variation in guard cells caused by and resulting in the opening and closing of stomata; variation or lack of variation in respiration with the pH of external media.

This leaves detailed investigations of particular cases to be carried out. It leaves practically untouched the possible internal variations with photosynthesis and respiration, the possible variation in photosynthesis of submerged plants with the pH of the medium which are still obscure in spite of the *Elodea*-chalk controversy and its more or less satisfactory settlement.

Variation in reaction of the natural vascular sap has been touched upon by BENNETT and his collaborators, but much more remains to be done in the investigation of the real pH, and the buffer systems if any, in the wood sap of trees and also in the fluids of the phloem and latex passages.

The problems of the connection, causal, correlated or merely concomitant, between the known variations in reaction -- which have been found to be associated with certain differences in times, stages of development, conditions, functions and phylogenetic positions — turn out to be mainly problems concerning not proteins and amino-acids but (1) the detailed distribution of enzymes throughout the plant; (2) the production and utilisation of carbon dioxide, concerning which much is known; and (3) the metabolic production and storage or utilisation of organic acids, such as citric, malic and oxalic acid in the cell-sap, and such as α hydroxy-behenic, ricinoleic and other fatty acids in the cell-wall, concerning which very little is known.

It is impossible to summarise briefly the present position but we may suggest a few general probabilities:

1. Changes in the hydrion concentration of cytoplasm beyond the range pH 5·0—6·2 would appear to have little relation to the *living cell.*
2. Variations in the hydrion concentration of the vacuolar sap may be important:
   (a) as indicating variations in cell metabolism, and
   (b) as affecting the rate of penetration of external and / or internal solutes through the cytoplasmic layer, but not necessarily affecting the permeability of the cytoplasm as such.
3. Variations in the reaction of the wall may be correlated with some of the variations in cell metabolism 2 (a).
4. The variations in organic acid metabolism are important and are amongst the least known phenomena of plant physiology.
5. The buffer complexes of the vacuolar sap appear to be the effective factors in many so-called iso-electric phenomena.

In conclusion we may also suggest a few interesting possibilities:

1. Variations in the hydrion concentration of cytoplasm within the range pH 5·0 to pH 6·2 and / or sap variations in a wider range may have important effects upon —
   (a) the colloidal condition, dispersion, hydrophilic properties, etc., of the cytoplasm;
   (b) the permeability of the cytoplasmic layer to electrically neutral substances, such as sugars, and neutral salts, and to other substances in the molecular (or ionic ?) condition;
   (c) the density (and sign ?) of the charge upon the external and internal surfaces of the cytoplasm.

2. Variations in the enzymic distribution and/or the hydrion concentration of the vacuolar sap may have important effects upon —
   (a) the character and quantity of organic acid which occurs in the vacuolar sap;
   (b) the character and quantity of fatty or pectic acid, pectate or fat, which is secreted externally by the cytoplasm to form either immediately or at a distance (cp. the oil ducts in sunflower and other plants, also HERKLOTS p. 265), the material for wall differentiation.
3. Variations in the acidity of the walls may yield valuable data concerning the identity and behaviour of the substances which help to enclose the cytoplasm and sap in plant cells and tissues.

APPENDIX I

EMDEN'S METHOD FOR PHOSPHATES AS APPLIED TO PLANT JUICES

1. Prepare a Gooch crucible with asbestos. Wash the asbestos filter freely with distilled water and dry in an oven to constant weight.
2. *Solution A*. Prepare a solution of strychnine nitrate containing 15 grammes per litre. This solution keeps well.
3. *Solution B*. Prepare a solution of ammonium molybdate containing 50 grammes in 150 cc. of solution. This solution should be moderately fresh.
4. Prepare a mixture of nitric acid and water (HNO_3 2 vols. plus water 1 vol.)
5. *Solution C*. Add one volume of Solution B to three volumes of the diluted nitric acid (4). This must be done very slowly and with constant shaking or stirring. Any precipitate formed should re-dissolve on shaking. As molybdic acid separates out on standing Solution C should be freshly prepared.
6. *Solution D*. This is the actual precipitating agent and, as it does not keep, it should be made up only when required for immediate use. It is prepared by adding three volumes of Solution C (ammonium molybdate-nitric-acid mixture) to one volume of Solution A (strychnine nitrate solution).
7. Using from 2 to 5 cc. of centrifuged or filtered expressed juice, add an equal volume of 10 % trichloracetic acid. This precipitates proteins. Filter (7).
8. Add to the filtrate (7) an excess of Solution D. The volume of Solution D necessary will vary with the phosphate content. A volume two or three times that of the filtrate has been

found sufficient in most of the cases investigated[1]). This precipitates the phosphates as a flocculent precipitate consisting of complex groups of molecules the weight of which is **28·24** times the weight of H_3PO_4 present. Collect the precipitate in the Gooch crucible, on the filter; wash it well with distilled water, using a filter pump, and dry in a water oven to a constant weight. The weight of H_3PO_4 in the sample of juice taken is obtained by dividing this weight by **28·24.**

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[1] The addition of more Solution D to the filtrate from the first phosphate precipitation is a precaution which it is wise to take, especially when the first precipitate is very abundant. Only one case has shown further phosphate with this test.

APPENDIX II

ORGANIC ACID ANALYSIS

Method of HILGER and CROSS[1]), as developed by C. T. INGOLD and J. I. ARMSTRONG.

The solution containing the free organic acids is divided into two parts which are treated as follows: —

I

Mix with alcoholic potassium acetate and half a volume of alcohol. Allow to stand for several days in the cold when the greatest part of the *tartaric acid* comes down as *potassium bitartrate.*

To estimate the tartaric acid this precipitate is dissolved in hot water and titrated with 0·1 N NaOH. The titrated liquid can be used for identifying the tartaric acid by adding calcium chloride to the solution and allowing the resulting filtrate to stand for some time when the calcium tartrate should separate out.

The filtrate from the potassium bitartrate is mixed with a few ccs. 10 % calcium chloride and is brought to a weakly acid reaction (pH 4·8—5·0, using BCG green to blue change) with lime-water and this precipitates the *oxalic acid* as *calcium oxalate*, which can be estimated by dissolving in dilute (about normal) sulphuric acid and titrating with N/10 $K_2M_nO_4$.

Filter off the calcium oxalate, neutralise the filtrate (pH 7·0—7·2 with PR) and allow to stand for some time. At this stage there separates out the remaining *tartaric acid* and *oxalic acid* as well as *citric acid.*

After filtration the entire precipitate is heated for ten minutes with dilute KOH solution which dissolves the *tartrate*[2]) while

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[1]) Landw. Vers. Stat. 33. 184. 1887, cited by CZAPEK, Biochemie der Pflanzen. Bd. III. pp. 98—99. 1925.

[2]) Practically all the tartrate comes down previously as bitartrate.

oxalate and *citrate* remain undissolved. The *citrate* is then dissolved in acetic acid while the oxalate, if any, remains behind. Oxalate, if any, should be titrated. Citrate is estimated by neutralising the acetic acid, filtering and weighing the precipitate as calcium citrate.

The neutral filtrate after the precipitation of the above three acids may still contain *calcium malate* and this can be precipitated by the addition of 2—3 vols. of alcohol, and weighed as calcium malate.

The alcoholic filtrate from the malate precipitation is treated with a solution of lead acetate. The resulting lead precipitate is decomposed with sulphuretted hydrogen and after removal of the lead sulphide by filtration the solution is treated with copper sulphate solutions and eventually *copper glycollate* may separate out.

The filtrate from the lead precipitation may still contain *succinic* and *lactic acids*.

The solution is evaporated to dryness, mixed with some HCl, and extracted with ether. The ether solution is evaporated, mixed with water and tested for the above acids. *Succinic* acid may be estimated as the barium salt (see C.R. 165, p. 793, 1917) and *lactic* acid in the usual way as the zinc salt.

II

The second part of the solution, utilised as a check, is neutralised with ammonia and boiled until crystallisation starts, or until the solution is sufficiently concentrated. Having neutralised, add two volumes of alcohol to one of the solution and allow to stand for several days. At this point there crystallises out the ammonium salts of *tartaric, oxalic* and *citric acids*. This precipitate (A) is dissolved in water and acidified with acetic acid, then potassium acetate and alcohol are added. After standing for two days the *bitartrate of potassium* which separates out is filtered off and titrated. The filtrate when mixed with calcium chloride may give a precipitate of *calcium oxalate*, which is separated, dissolved and titrated. The filtrate from the oxalate is neutralised with lime water and an equal volume of alcohol added in order to precipitate the *calcium citrate*, which is weighed.

The mother solution of precipitate A may contain *citric acid* and *malic acid*. The solution is neutralised with lime water and, after adding calcium chloride, is heated in order to precipitate the *calcium citrate,* which is weighed and added to the previous quantity[1]). Excess alcohol added to the filtrate from this calcium citrate precipitates the *calcium malate,* which is weighed.

JORGENSEN carried out the difficult quantitative separation of *citric acid* and *malic acid* in this way: —

The solution containing the two acids is brought to a faintly alkaline reaction, then the barium hydroxide and 28 % alcohol are added. The precipitate formed is *barium citrate* and is thus separable from the much more readily soluble *barium malate.* This method is not reliable in the presence of calcium which precipitates as a mixture of citrate and malate on neutralisation.

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1) This part of the citrate may be proportionately quite large.

N. B. All quantities are small and require small scale apparatus together with meticulous care, in order to get even a 90 % accuracy.

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APPENDIX III

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KNOTT, J. E. 1926. Catalase activity in relation to growth. Cornell Univ. Agric. Exp. Station. Memoir **106**, Ithaca. Observations on spinach plants include various values for the leaf juice from pH 6.0 to pH 6.4, for the apical two inches of seed stalk from pH 6.0 to pH 6.5. H-electrode method was used and no relation was found between this (residual) pH of the juice and the catalase activity of the intermediate leaves.

MEVIUS, W. 1927. Kalzium-Ion und Wurzelwachstum. Jahrb. wiss. Bot. **66**, 2, 183. With *Pinus pinaster* roots, KCl or $MgSO_4$ solutions limited root development at pH 3.3—4.3; above pH 7.0 injury was found with either alone but also antagonism with mixed solutions. This includes a discussion of IEP and calcium.

TOGASHI, K. 1928. On the development of two races of *Valsa* in relation to the H. i. c. of Peach trees. Agric. and Hort. **3**, 893. ex Jap. Jour. Bot. **4**, 3, 76. Data include -- pressed juice of "healthy peach trees" about pH 5.0; cortex 4.4—5.6; medullary rays in bast 4.4 —5.0, in cambium 4.4 -4.6; bast 5.0 -5.8. Culture experiments show optima for Race A pH 5.5, Race B pH 6.0. In diseased trees the h. i. c. is higher than normal, e. g. pH 4.4 4.6 or even pH 3.6 3.8 in some cases. An "isometabolic point" (SIDERIS) could not be found.

CHODAT, R. et SENGLET, A. 1928. Sur le Sapécage du Maté. Arch. Sci. Phys. et Nat. (Genève) **5**, 10, 55. Data include fresh leaf juice of *Ilex paraguariensis* pH 4.6; water extract of dried leaf pH 5.4; extract of sapequée leaf pH 7.14.

KACZMAREK, A. 1929. Untersuchungen uber Plasmolyse und Deplasmolyse in Abhangigkeit von der W. i. k. Protoplasma **6**, 2, 209. Present plasmolytic methods are not sufficient to solve the problem of the relation of cell permeability to the pH of the external medium. Maxima are reported to occur at pH 4.2, also between pH 5.5 and pH 7.5; minimum in the range pH 4.3 -5.5.

PFEIFFER, H. 1929. Der isoelektrische Punkt (IEP) und die aktuelle Azidität von meristematisierten Zellen. Protoplasma **6**, 3, 377. A careful and critical survey of all the factors noted. The actual pH of the cambium is sometimes even further away from the true IEP of the plasma

colloids than is the pH of the other stem tissues. The apparent IEP is shown to be really an equilibrium point, somewhat as described above in Chapters XVI—XX. The R. I. M. is used and the R. I. M. results given above are confirmed as distinct from ZACHAROWA's high values, thus —

	Phloem	Cambium	Xylem
Cochlearia armoracia. . .	5.8 6.0	5.6 6.0	- 4.6
Ligustrum vulgare. . . .	4.8 – 5.8 (4.5)	5.4 6.0 (4.6)	4.4 4.1 (3.9)
Rhododendron maximum.	5.2 – 6.0 (4.7)	5.4 6.0 (4.9)	4.4 3.6 (3.8)

( ) probable IEP determined by dye uptake minima. This author concludes very wisely that in the fixing of an equilibrium point other factors as well as pH are effective. Still another theory of cell growth and differentiation is also considered.

PFEIFFER, H. 1929. Der isoelektrische Punkt von Zellen und Gewebe. Cambridge Biological Reviews 4, 1. Biological work on IEP has dealt, not with the true IEP, but with an apparent IEP which depends primarily upon the reaction at which there is a maximum of neutral molecules. Compare Chapters XVI XX above; even this generalisation may not apply to some of the socalled isoelectric point data, e. g. to observations which are really concerned with the buffer complex of the *sap* in relation to external fluids.

PFEIFFER, H. 1929. Elektrizität und Eiweiße. Dresden. Proteins and their electrical properties as amphoteric colloids are considered in detail, including electrical changes with variation in pH.

DAVIS, E. F. 1929. Some chemical and physical studies on the nature and transmission of "Infectious Chlorosis" in variegated plants. Ann. Missouri Bot. Garden 16, 145. Electrometric determinations of h. i. c. were made by the quinhydrone electrode method, modified to accommodate determinations made in single drops of expressed juice.

KELLER, R. and others. 1929. Elektrostatik in der Biochemie. Kolloidchemische Beihefte, Sonderausgabe 28, 7–10. Vorträge des Kurses in Basel. Oktober 1928. Dresden.

ARMSTRONG, J. I. 1929. Hydrogen-ion Phenomena in Plants. (A new series about to appear in Protoplasma 8).

I. Hydrion Concentration and Buffers in the Fungi. Thesis Q. U. B. An account of the tissue reactions of various fungi (see p. 95 above), together with detailed analyses of the buffers present in selected species (see p. 361 above). The buffer complex of *Collybia velutipes* would appear to be more or less completely analysed. The data given are more up-to-date than those above.

II. An investigation of the Buffer Complex of Sap from the Stems of *Pelargonium* sp. Thesis Q. U. B. A detailed analysis of the buffer

systems constituting the buffer complex in the stem of *Pelargonium* sp., which includes the following —.010 M phosphate; .0537 M oxalate; .0337 M malate; .0066 M tartrate; .003 M citrate and a small quantity of an unidentified hydroxide; explaining from 80 to 100 per cent of the buffer index of the sap in the range pH 3.0—7.0.

III. The Acidity of certain cell-walls considered in relation to the higher fatty acids. MS. Q. U. B. A systematic survey of the reactions of the higher fatty acids with the R. I. M. indicators; the acids used include — pelargonic, capric, lauric, myristic, palmitic, margaric, stearic, arachidic; tiglic, oleic, erucic, linoleic, ricinoleic and dihydroxystearic. These show various degrees of "base-avidity" in relation to indicator acids, and the phenomena observed furnish a reasonable explanation of the colorations obtained with the R. I. M. used on lignified, suberised or cutinised cell-walls.

Buxton, B. H. and Darbishire, F. V. 1929. The pH Value of Cell Sap of Flowers. I and II. Jour. Roy. Hort. Soc. 54, 1.

I. Deals with pH values obtained by macerating dried petals in distilled water for various periods.

II. Deals with pH values obtained with a B. D. H. Capillator on the expressed juices of white petals. Two average initial points at pH 6 and at pH 5.6 are given. Data for fresh sap of whitish petals -- *Rhododendron* 4.0; paeony 4.5; poppy (Shirley) 4.5; lupin 4.6; rose 4.7; *Aquilegia* 4.8; primrose 5.0; *Iris Kaempferi* 5.0; *Campanula* 5.5.

Buxton, B. H. and Darbishire, F. V. 1929. On the behaviour of "Anthocyanins" at varying H. i. c. Journ. of Genetics 25, 1 (April) 71. A valuable contribution – yellow and the yellow element of green in petals are traced to flavones which go yellow above pH 8; two groups of anthocyanins are distinguished; both of which are red at pH 3 and pinkish up to pH 5: the "blue" type gives a mauve about pH 6 and clear blue at pH 7, passing to greens above pH 7 if flavones are present: the "red" type never shows blue but passes to red again at pH 7 and to browns above if flavones are present. Many examples are given of typical "blue" and typical "red" flowers, together with intermediates where both elements are present in varying proportions. *Anagallis* is aberrant.

Svedberg, The. 1929. Mass and Size of Protein Molecules. Nature 123, 871. All *stable* native proteins so far studied show two groups:

1. haemocyanins with molecular weights of the order of millions; e. g. from blood of *Helix pomatia* 5.000.000 and 12 $\mu\mu$ radius, and from *Limulus polyphemus* 2.000.000 and non-spherical.
2. all other proteins with mol. wts. from about 35.000 to 210.000; showing four sub-groups:
   (a) spherical, e. g. ovalbumin, Bence-Jones' protein;
   (b) non-spherical, mass 2a, e. g. haemoglobin, serumalbumin;

(c) non-spherical, mass = 3a, e. g. serum globulin;

(d) spherical, mass = 6a, e. g. Rhodophyceae-phycocyan, Cyanophyceae-phycocyan, Rhodophyceae-phycoerythrin, edestin, excelsin, amandin.

Molecules of sub-group (d) are disaggregated with increasing pH, passing to sub-group (c) wholly or in part when the pH is changed from 4.6 to 6.8, or to sub-group (a) at pH 11–12. Edestin is stable from pH 5.5 to pH 10; at pH 11.3 some molecules are disaggregated to sub-groups (b) or (c).

(See pp. 1 and 322–3, VLÈS' suggestion of *unstable* protein complexes varying with changes in pH is strengthened by this recent work.

BROOKS, M). M. 1929. Studies . . . . . X. The influence of experimental conditions upon the penetration of methylene blue and trimethyl thionine. Protoplasma 7, 1, 16. The total penetration of methylene blue into the cells of *Nitella* and *Valonia* is again found to be independent of the pH of the external solution. (See also Proc. Nat. Acad. Sci. 13, 12, 821. and Univ. Calif. Pub. Zoology 31, 6, 79.) The *rate* of penetration is, however, now found to vary with the external pH, being less rapid at pH 5.5 and more rapid at pH 8.83 (See pp. 314–317 above).

BROOKS, M. M. also has a series of "Studies on the Permability of Living and Dead Cells" in Public Health Reports, U. S. P. H. S., Washington; Reprints Nos. 845, 846, 866, 888, 986; which it is hoped will be made more generally available in a forthcoming volume of Protoplasma Monographien.

I. 845. Deals with the penetration of acids into *Valonia*. The natural sap had pH 6.2–6.4, or pH 6.6–6.8 with free CO_2 removed, or even pH 8.4 with further aeration. HCl and HNO_3 lower the sap value to pH 5.2 by liberating CO_2.

II. 846. Deals with the penetration of alkali bicarbonates. These also decreased the sap pH to 5.2–5.4, $NaHCO_3$ acting more rapidly than $KHCO_3$, but the pH of the CO_2-free sap was *increased* more rapidly by $KHCO_3$ than by $NaHCO_3$, indicating a more rapid penetration of the K-ion.

III. 866. Deals with the penetration of alkalies and ammonium salts. NH_4OH, NH_4Cl and $(NH_4)_2SO_4$ raise the sap values to above pH 8 in 20–60 minutes; $(NH_4)_2CO_4$ raises it to about pH 7.5 in 20 mins. The toxicity of the first three decreases from left to right. $NaOH$ and KOH have very little effect upon the internal pH, even after 90 mins.

IV. 888. Deals with the penetration of arsenic into wall, protoplast and sap of *Valonia*; the protoplast takes up most and the quantity increases with divergence from neutrality (externally) in both directions. Phosphate buffers have a strong effect, decreasing penetration markedly.

'HCO_3 or CO_2 increases the penetration of pentavalent arsenic into the protoplast by 150 per cent and decreases the trivalent arsenic by 25 per cent, but the penetration into the sap is increased about 100 per cent for As_2O_3 and about 25 per cent for As_2O_5.

V. 986. Deals with the effects of $NaHCO_3$ and NH_4Cl on arsenic penetration. A minimum is again found at neutrality in the external solution for both protoplast and sap. Decrease of internal pH with CO_2 ($NaHCO_3$) gives increased penetration with pentavalent arsenic for both sap and protoplast, but with trivalent arsenic an increase for the sap and a decrease for the protoplast. Increase of internal pH with NH_3 (NH_4Cl) gives with pentavalent arsenic decreased penetration for both sap and protoplast, but with trivalent arsenic an increase of penetration for both sap and protoplast. (See and compare the remarks at top of p. 319 above.)

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The following occur in Appendix III

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